CORN ROOT ROT STUDIES

by

B. B. BRANSTETTER, B.S. in Agr.

SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

in the

GRADUATE SCHOOL

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The experimental data reported in this paper show that most of the kernels on practically every ear of corn grown in Missouri are internally infected with one or more of the following organisms: *Diplodia zeae*, *Fusarium moniliforme*, and *Cephalosporium acremonium*. Yield tests comparing heavily infected and lightly infected seed show that reduction in yield from planting heavily infected seed is due to reduced field stands caused by seedling blight and not to corn root rot. By increasing the planting rate of heavily infected seed over lightly infected seed so that nearly equal stands were obtained, the resultant yields from both lots of seed were made nearly equal. The employment of certain physical ear characters in selecting lightly infected seed corn was found to be more practical than the germinator method. Inoculation trials carried on in the field and in the greenhouse with *Diplodia zeae*, *Fusarium moniliforme*, *Cephalosporium acremonium* and *Gibberella saubinetii* showed these organisms were capable of producing a certain amount of seedling blight but not corn root rot, which develops as the corn plant nears maturity. A Pythium-like organism isolated from diseased corn roots was used to inoculate disease-free seedlings grown in uninfected soil in 1927. Typical corn root rot resulted only in the early plantings. From these diseased corn roots the organism used for inoculation was reisolated in pure culture. Corn root rot in Missouri is probably caused by a soil-borne Pythium-like fungus.

ACKNOWLEDGMENT

The experimental work reported in this thesis has been conducted in the Department of Botany of the University of Missouri with the Cooperation of the Department of Field Crops. The writer wishes to express his appreciation to Dr. W. J. Robbins for encouragement, advice and assistance throughout the investigations and to Dr. E. F. Hopkins for suggestions and assistance in the earlier phases of the work. The writer is also deeply obligated to Dr. W. C. Etheridge and Professor C. A. Helm for suggestions, criticisms and assistance in carrying out the work.

Corn Root Rot Studies

B. B. Branstetter

Corn root rot is a term used loosely for a number of more or less well known parasitic and non-parasitic diseases of corn which include corn root, stalk and ear rot diseases. Holbert and his co-workers, in a recent publication, stated: "For brevity, these diseases are sometimes called 'corn rot diseases' and frequently simply 'corn root rot'. However, at the outset, it must be realized that 'corn root rot' is not one disease, but several diseases, some of which do not result in any rotting of either roots or stalks." Thus one realizes that the corn root rot problem at the present time is a complicated one and sufficient attempts have not been made to separate the different diseases and to evaluate properly each of them.

In the earlier work on the problem, particularly by Hoffer and Holbert[43,55] at Indiana and Illinois respectively, the selection of good seed corn was emphasized as the most important factor in controlling and preventing corn root rot. It was suddenly realized that nearly every ear of corn was infected with one or more fungous organisms and by special methods of germination the least infected ears, which could be planted with no harmful effects from that source, could be separated from the others. The connection between infected seed corn and corn root rot was taken for granted, and, at first sight, was quite apparent. But confusion arose when seedling blight and rotting of full grown plant roots were considered to be due to the same causes. So much attention was given the seed-borne pathogene phase of the problem that the organisms directly responsible for and associated with the rotting of roots on full grown plants were studied very little. Seedling blights either killed the plant in the seedling stage or stunted it so that in many cases it never fully recovered, and thus produced an inferior plant with a small ear or no ear at all. So much stress was given to this idea that in much of the earlier literature delayed development after seedling blight injury was considered to be typical corn root rot. Valleau[118] rightly points out, in this connection, that; "If these effects of seedling blight are to be considered corn root rot, the fact should be recognized. If however, there is a true corn root rot other than seedling blight, the two diseases should be recognized clearly and a distinction should be made between them in future literature."

There is unquestionably a disease, or group of diseases, that causes seedling blight of corn. This disease should be called corn seedling blight

Note.—Also submitted to the Faculty of the Graduate School of the University of Missouri as a thesis in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

since its connection with the rotting of corn roots as the plants reach maturity is not well established. Holbert[58] and others have shown that when seed known to be infected with *Gibberella saubinetii*, *Diplodia zeae*, or possibly others of the common corn ear pathogens is planted, lower yields result than if apparently healthy seed is used. Also diseased seeds are known to produce weak plants which may die in the seedling or any other stage before reaching maturity, or which may be weak throughout their lives. Many practical farmers have known for a long time that poor stands and resultant yields may come from the use of diseased seed and have been careful to select ears free from visible evidence of true ear rot organisms such as *Diplodia zeae*, *Gibberella saubinetii*, and *Fusarium moniliforme*. By selecting such ears in the field in the fall from sound stalks and curing them properly, it is possible to secure seed corn that produces practically no seedling blight due to kernel infection. Seed corn selected in such a manner is not by any means free from seed-borne organisms; in fact, results obtained in the course of this investigation show that nearly every kernel in most corn ears is infected with one or more species of fungi. However, good seed corn which shows in a germinator a high percentage of germination and little infection produces in a normal environment seedlings that are able to develop without being appreciably affected by the presence of fungi in the seed.

Corn root rot, however, may develop later in the season even though there have been no signs of seedling blight in the early stages of the development of the corn. It is this fact that leads the writer to consider seedling blight and root rot two entirely separate and distinct diseases. On the Missouri Experiment Station field at Columbia, where many of the experiments in this investigation have been carried out, evidences of corn root rot do not appear in the field until about the time of ear formation. At this stage the roots begin to rot badly so that in a short time a fully developed stalk may be rather easily lifted out of the soil, all of the main roots being rotted off at the depth of three or four inches. It is this condition which causes the corn to go down badly just before maturity especially if the soil is softened by rains that are accompanied with wind (see Plate 1). These same symptoms are exhibited by corn all over the state if we may judge by a few personal observations of he writer and by descriptive letters and diseased corn specimens accompanying them sent by farmers to the Department of Botany of the University of Missouri. If this condition then is to be recognized and considered as true corn root rot in contrast to seedling blights and poor development due to adverse soil conditions, we must not overlook the fact that there is another disease of corn described by Hoffer[46] that exhibits much the same symptoms as corn root rot and is probably non-parasitic in nature. According to Hoffer[50]: "It is difficult, at times, to distinguish between

the symptoms displayed by the plants which are due to root rots and those resulting from low or unbalanced fertility (malnutrition). Soil conditions causing malnutrition are often due to a lack of but one of the essential plant food materials. They may result from the use of unbalanced fertilizers. Soil erosion, leaching of soils, changes in level of water tables, thin surface soils, lack of drainage, competition of weeds, and other factors contribute to cause malnutrition of corn plants." Although a number of organisms, especially *Fusarium moniliforme* and *Gibberella saubinetii*, that cause seedling blight, are always present in the root and stalk lesions, Hoffer considers them entirely secondary and saprophytic in nature.

Early in the course of the present investigation, the writer realized that the true relation between root rot, stalk rot, ear rot, and seedling blight must be determined before real progress could be made in solving the so-called corn root rot problem. Hence, one of the principal lines of investigation was to determine the relation of corn seedling blight, which occurs early in the life of the plant to corn root rot, which occurs normally late in the life of the plant. In this paper evidence will be given to show that there is a disease of corn roots quite distinct from seedling blight and its after effects and that the organisms generally considered to be the cause of corn root rot are probably not concerned in the problem. In arriving at this conclusion, investigations were carried out on the extent and nature of natural fungous infection in the seed corn in Missouri, on the effect of seed infection on yield under Missouri conditions, on the relation between seed infection and true corn root rot, and on the cause and prevalence of corn root rot as it normally occurs in Missouri.

REVIEW OF THE LITERATURE

References to corn root rot by plant pathologists are comparatively recent though the occurrence of certain organisms causing kernel and cob rots have been noted from time to time since 1904. The first important disease of corn to be described in this country was Stewart's disease or bacterial wilt. Stewart[107] described the disease in 1897 saying it was caused by bacteria. He sent a culture of the causal organism to E. F. Smith[105] who named it *Pseudomonas stewartii* in 1898. The organism is now generally referred to as *Bacterium stewartii*. It has never been considered as one of the corn root, stalk, or ear rot pathogens.

Burrill[7] found a bacterial disease of corn to be quite serious in many fields in Illinois and in a bulletin published in 1889 stated: "In anything like severe cases at least one-half the roots—always the lowest—are injured and usually dead. The bottom portion of the stalk is likewise affected and will be found dead or dying." It appears that the trouble Burrill described must have been due to something other than bacteria

since no one has confirmed his original work. Burrill himself never named the organism which he considered the cause of the corn disease he observed, but according to Rosen[96] the name *Bacillus zeae* Burrill was given it in 1892 by Russell[97] who probably for convenience adopted a name and used for authority the name of the man from whom he had obtained his cultures. Rosen[94,95], while working on another bacterial disease of corn, examined the specimens left by Burrill at the University of Illinois and found them to show various blade and sheath spots, quite comparable to the spots described by Durrell[12] and attributed to *Diplodia zeae*.

Rosen[94] described a bacterial root rot of field corn in 1919 which he considered at the time to be identical with the disease reported by Burrill in 1889. In a more recent paper, however, Rosen[96] described the disease as a stalk rot only and named the causal organism *Phytomonas dissolvens* (comb. nov.). It was in this latter publication that he mentions having examined Burrill's specimens and considers the disease described by Burrill to be in no wise similar to the disease produced by *Phytomonas dissolvens*. The disease has been observed in Arkansas and many other central states for a number of years. The disease is considered to be serious in Arkansas only during those periods of the corn growing season when the temperature, rainfall, and humidity are above normal. In the absence of high temperatures and humidity, the disease is found to be entirely absent or of minor importance. It is described as "primarily a disease affecting the stalk and leaves exhibiting the following symptoms: (1) a light or dark brown rotting of bases of leaves, particularly those at the base of the stalks; (2) a rotting of the lower portion of the stalk, the affected parts being dark brown, soft, putrid, and sunken in fresh infections. These may extend through the entire width; or, as more frequently found, remain localized as dark, rotted spots with margins appearing water-soaked. The disease consists of a localized necrosis of parenchymatous tissue."

The first mention made in the literature of organisms now considered to be connected with the corn root, stalk, and ear rot problem was by Sheldon[101] in 1904. He described a new species of fungus, *Fusarium moniliforme*, found on ears of corn from many farms in Nebraska. At that time the pink growth formed by *Fusarium moniliforme* on the ears of corn was thought to be the cause of certain diseases of livestock, particularly "staggers" in cattle, but Sheldon attempted to do no more than describe the organism and its appearance on corn ears.

The first suggestion made in the literature that *Diplodia zeae* is a parasite on corn was made by Heald[40] in 1906. He stated in a paper entitled: "New or Little-known Plant Diseases in Nebraska," that: "Moldy corn is often due to a fungus provisionally referred to

Diplodia maydis, but differing in several points in habit and structure." In a later publication Heald[41] and his co-workers described the same fungus under the name of *Diplodia zeae*, which name has been universally adopted. Burrill and Barrett[8] discussed four ear rots of corn causd by *Diplodia zeae*, Fusarium I, Fusarium II, and Fusarium III. Diplodia was estimated to be responsible for 90% of the ear rots due to parasitic fungi in Illinois. Infection originated, they found, from wind-borne spores of all four organisms. Ears were never affected by means of anything working up through the stalk, but always from spores lodging in the husks of the ear. Carman[35] observed in Kentucky that "a pink mold (a fusarium) is very common in our fields and causes many grains apparently sound to assume a pink color." He also noticed that "what appears to be the same fungus is found on corn that is germinating badly in the field." He advised that "such corn ought not to be planted." He[36] assumed that the elimination of the discolored kernels for seed would eliminate the infected ones and advised that show corn bearing pink kernels should be eliminated from any corn contest. He believed that the pink mold "gets to the kernels only by way of burrows made by the corn-ear worm and would thus probably be controlled by preventing injuries of the insect." Diplodia, on the other hand, "remains in the field and continues developing there in the grain and stalks long after the crop is cut. Old stalks lying about fields may continue to produce spores of the fungus for several seasons," and thus infect growing ears by gaining entrance particularly through the shank end. Selby[98] reported that a dry-rot or mold of corn was investigated at the Ohio station in 1906. He stated that a grower in Licking County "reported that conditions clearly indicate the invasion of the soil by the parasite and possible infection through the growing plant."

It was not until 1910 that fungous organisms were noted as parasites on corn roots. In an addendum to his bulletin entitled "Diseases of Cultivated Plants," Selby[99] calls attention to a root rot of corn "attributed to a species of Fusarium of similar behavior to the scab of wheat, potato fusarium, and cabbage wilt, in that the parasite appears to survive in the affected soil, as well as elsewhere." Reports from Fayette County stated that diseased corn roots occurred in "patches where the stalks blow over easily, and rarely form mature ears. Roots of such plants are partly killed and thus weakened." The advisability of practicing crop rotation to control the disease was suggested. Pammel[85] briefly described corn root rot in the Iowa Agriculturalist in the fall of 1914 and during the following spring issued a press bulletin[86] on the subject from the Iowa Agricultural Experiment Station.

The first comprehensive piece of work published on corn root, stalk, and ear rots is that of Pammel, King and Seal[87]. The disease, which was

attributed to a species of fusarium, apparently became serious in Iowa for the first time in 1914 when it caused an estimated loss of $15,000,000 to the corn crop. One of the authors observed the disease in Missouri and Illinois that year, and its occurrence was reported from Nebraska and Minnesota. The fusarium disease was described as attacking the roots, the stalks, and the ears of corn, at least in some seasons; however, it was not determined whether or not all these symptoms were caused by the same organism. It was thought that the disease spread largely with the seed corn. The following recommendation as a preventive measure was made: "Careful seed selection is a good measure of precaution; in no event use seed corn that comes from a diseased field. The most feasible line of preventive work will be the development of resistant varieties."

Hewitt[42] reports that in Arkansas "ear molds caused by Fusarium are serious," constituting "the limiting factor in the production of some varieties." The affection is believed often to follow ear-worm injury but may be quite serious independently.

Selby[100] called attention to the fact that corn root rot was widespread in Ohio in 1918. In describing the disease he stated: "The diseased plants may be purplish-colored, dwarfed or stunted, and unproductive. The plants may show dying at the top or general lack of deep green color. The disease causes a rotting of the roots, the fungus extending upward into the stem of the corn plant, showing as a darkening of the joints in the stem; in severe cases the fungus extends upward, entering the ear of corn through the ear shank and making a final possible development as a pink mold of the ear. Growing corn is studied by lifting and cutting open the stem from the main root upward." He considered the causal organism to be one or probably more soil-borne species of fusarium. Since the soil was thought to be quite generally infected with these fusaria, he suggested crop rotation and development of resistant strains of corn as the best means of control.

About the time Selby found corn root rot prevalent in all parts of Ohio, the disease became generally recognized by plant pathologists and farmers alike as being more or less serious all over the corn belt. As a result the office of Cereal Investigations of the Bureau of Plant Industry, U. S. Department of Agriculture, organized in 1917 the "Corn Root, Stalk, and Ear Rot"project with G. N. Hoffer, at LaFayette, Indiana, in charge of laboratory and field investigations. In the first project publication, Hoffer and Holbert[43] considered species of Gibberella, Fusarium, Verticillium, Rhizopus, and Pseudomonas to be the harmful organisms responsible for the root, stalk, and ear rots. They state: "The planting of seed infected with these organisms is, in a great measure, responsible for missing hills, slow-growing stalks, barren stalks, downstalks, nubbins, and early blighting of plants in the field with the large

reduction in yield which these conditions bring about." Two types of infection are described. Primary infection takes place from an infected seed which was produced on a diseased plant; secondary infection takes place later in the growing season from the organisms which live in the soil on old plant remains or which have been carried into the soil on the infested seed which was used for planting. The inter-relation of wheat scab and corn stalk and ear rot produced by *Gibberella saubinetii* was suggested when they state that wheat planted in fields of diseased corn has more scab than occurs when the corn fields are free from the scab-producing organism. They recommend that for seed corn purposes no infested and weakened ears should be planted. They explain how such ears may be detected by growing representative kernels on a special type of germinator. After the seedlings have grown to a height of three or four inches it is suggested that "those seedlings which have rotted embryos and stalks indicate the ears to be discarded for seed purposes. By reading the germinator on the basis of these rotted seedlings, and eliminating all of the ears which show this rot on the germinator, the primary infections which would otherwise occur in the field from seed from such ears and which would considerably reduce the yield in the field, can be prevented." In a later paper they[51] state that "corn generally is more or less affected by root and stalk rots. Probably the greatest loss due to these rots is caused by using infected seed. The common wheat scab organism, *Gibberella saubinetii* (Mont.) Sacc., is probably the most common pathogene responsible for much of the root and stalk rotting of corn plants in the Central States." The evidence on which this statement was based is not given in their paper. Hoffer[45] pointed out that methods similar to those employed in the selection of disease-free field corn should also be used for sweet corn which is similarly affected by the root rot. In larger plantings of sweet corn, where it is practically impossible to germinate seed from each ear, he suggested the elimination of any showing physical defects, particularly brown discolorations of the seed.

Valleau[116] reported that corn fields examined about Lexington were found to be infected with root and stalk rots to the extent of nearly 100 per cent. He examined hundreds of ears of corn from Kentucky and other states for seed-borne organisms and found every ear to be infected with *Fusarium moniliforme*. In studying the control of root rot he found the seed germinator method of selecting disease-free seed corn was not practical as a means of eliminating diseased ears. It was of value only in eliminating some of those ears so badly infected that plants from them succumbed to seedling blight. He found that Gibberella species may or may not be present in a field badly infested with root and stalk rots. Since *Fusarium moniliforme* appeared to be a more active parasite when

it and Gibberella species were associated on rotting stalks of corn, and since seed corn was infected to such a high degree with *Fusarium moniliforme*, he considered it the most important cause of root and stalk rots of corn.

Duddleston and Hoffer[25] reported that in a test of over 14,500 ears at Shelbyville, Indiana, in 1920, twenty-seven per cent showed serious infections of Fusarium species and Diplodia.

Sherbakoff[102] in his investigations found that "in Tennessee, as in other states, the most common Fusarium of corn is *Fusarium moniliforme* Sheldon."

Clayton[15] reported that work done in Ohio during the winter of 1920 and 1921 showed that the fungus, *Diplodia zeae*, was very prevalent in seed corn.

Manns and Adams[75,76] reported four parasitic fungi as very common in seed corn. In an isolation study they[76] found that Delaware corn was internally infected with *Cephalosporium sacchari*, *Gibberella saubinetii*, *Fusarium moniliforme* and *Diplodia zeae* to the extent of 39.54 5.95, 19.92, and 5.69 per cent, respectively. In a later paper Manns and Phillips[77] determined the parasitism of these fungi by inoculating soil in which corn plants were grown under sterile conditions in the greenhouse. They found that *Gibberella saubinetii* was the most active seedling parasite of corn and suggested that it may be an important factor in reducing stands. *Fusarium moniliforme* appeared less active as a seedling parasite than *Gibberella saubinetii*. *Diplodia zeae* was found to be quite active in retarding the growth of the young plants, while *Cephalosporium acremonium* showed no pathogenicity whatever in their studies.

Taylor[111], Chief of the Bureau of Plant Industry, United States Department of Agriculture, in reporting on the rots of corn for 1921 stated that "The chief fungous parasites are *Gibberella saubinetii* (the wheat scab fungus), different species of Fusarium, especially *Fusarium moniliforme*, and *Diplodia zeae*."

Tehon[112] states that "Root and stalk rots of corn are chiefly caused by the fungi *Fusarium moniliforme*, *Diplodia zeae*, and *Gibberella saubinetii*. The degree of injury done by these organisms is dependent upon the relative susceptibility of the corn plant in the various stages of its development, and the effects of the attack appear as rotted roots, barren stalks, and poorly filled ears."

Holbert[58] and his co-workers are apparently in accord with these statements in regard to the cause of corn root rot as their principal line of attack has been a study of the seed-borne organisms and their effect on the seedling plant and its subsequent development. Their work deals primarily with *Gibberella saubinetii* and *Diplodia zeae*, and the effect of the various environmental factors on the development of plants from

seed infected and not infected by these organisms. They conclude that "Planting of Diplodia-infected and Gibberella-infected seed always resulted in a reduced stand, many blighted and weak plants, and a lowered vigor and vitality of those plants which survive." Concerning the significance of *Fusarium moniliforme* they report that "Although extensive field inoculation studies have failed to yield definite data concerning the pathogenicity of this organism, the planting of seed primarily infected with *Fusarium moniliforme* has consistently resulted in a reduced yield of sound corn."

They recognize the importance of soil infection in the problem for they say: "Of the environing factors affecting the development of the different corn rot diseases under discussion in this bulletin, the problem of crop rotations and crop sequence takes a place second to no other under conditions existing at the present time in Central Illinois." That *Gibberella saubinetii* is considered of much importance in soil contamination is suggested by the statement that "In the experiment at Bloomington in 1920, there was a difference of 14.1 bushels in acre yield between corn from good seed and from scutellum-rotted seed where corn followed clover, but in the same field where corn followed badly scabbed spring wheat, the difference was 33.1 bushels."

The Plant Disease Reporter[88] gives *Gibberella saubinetii* as the cause of corn root rot.

Thus from the literature here reviewed, it is seen that there are four organisms, namely: *Diplodia zeae*, *Gibberella saubinetii*, *Fusarium moniliforme*, and *Cephalosporium acremonium* generally described by investigators as the most important ones associated with corn rot diseases.

Reddy and Holbert[91] were among the first investigators to make an attempt to separate the various diseases concerned in the corn root, stalk and ear-rot problem. Corn plants from seed infected with *Cephalosporium acremonium* were found to develop characteristic symptoms particularly blackened fibro-vascular bundles, purpling, prolific stalks, barren stalks, nubbin ears, and excessive suckering. They found this organism, which is carried internally in the seed, to produce no root rot whatever. They state, "It develops with the germinating kernel and causes a systemic infection of the plant through the vascular system. By this means, it invades the ears and eventually the kernels. In this manner it is carried over to the following season." Because of its most characteristic symptom the disease was called "the black-bundle disease of corn."

Hoffer[46,47] and his associates working in Indiana have attacked the corn root, stalk and ear rot disease problem from a different angle than have Holbert and his co-workers at Illinois. In a paper published in 1923, Hoffer and Carr[46] apparently attempt to show that corn plants are

attacked by the various rot diseases only when the soil is deficient in essential nutrients, or contains unbalanced combinations of available salts for absorption by the plants. Diseased plants were found to have an excessive accumulation of iron and aluminum in the nodes, which became brown, yellowish brown, and brownish purple in color and began to disintegrate in the absence of any specific organisms. Such a disturbance was thought to predispose the corn stalks and roots to attacks by various fungi including those generally thought to cause root and stalk rots. Later Hoffer and Trost[47] concluded that "accumulations of aluminum in corn plants are associated with retarded growths and increased susceptibility of certain strains to root rots, and when iron compounds gradually accumulate in the nodal tissues of the plants, the growth of the stalks may be little affected, but the disintegrations of the nodal tissues are accompanied by increased susceptibilties of the plants to root rot." More recently Hoffer[50] differentiates between corn root rot caused by disease organisms and a non-parasitic disease produced by accumulations of iron and aluminum in the plant tissues when he says "it is difficult, at times, to distinguish between the symptoms displayed by the plants which are due to root rots and those resulting from low or unbalanced fertility (malnutrition)." The common symptoms of malnutrition are described as: "light, yellowish-green leaves and stalks when sufficient nitrogen is lacking; or prematurely dead stalks; plants with chaffy ears borne on prematurely broken shanks and large stalks with badly rotted roots when sufficient potassium is lacking; plants with normal green leaves but stunted in growth, and poor stands in fields with acid soils when sufficient phosphorus is lacking." He recommends testing corn stalks chemically to determine their plant food needs. When corn stalk internodal tissue shows no accumulation of nitrate with the diphenylamine test the available nitrogen is said to be deficient in the soil; when nodal tissue shows excessive iron accumulations with the potassium thiocyanate-hydrochloric acid test the available potassium is deficient; and when plants are stunted on acid mineral soils containing sufficient nitrogen and potassium in available forms, the elements phosphorus and usually calcium are said to be deficient.

The Ohio Experiment Station has taken exception to Hoffer's corn stalk test method of determining plant food needs. In a bulletin[122] published five months after Hoffer's bulletin describing the tests, Salter, Chief of Agronomy at Ohio, states that "Farmers of the State are advised against the use of the method as a guide in choosing fertilizers of the corn crop." This statement was based on the evidence secured by Welton, Morris, and Gerdel in testing more than two thousand corn stalks from plots whose fertility requirements were known. These tests were made in different sections of Ohio during the summer of 1926. They found that

"the corn stalk tests" were extremely variable, "and therefore impossible to interpret, frequently conflicting and often misleading and unreliable."

Valleau[119] has recently described corn root rot as a soil-borne disease produced by a Pythium-like organism that he was unable to isolate in pure culture. From artificial inoculation studies he found that *Gibberella saubinetii* and *Fusarium succisae* could produce seedling blight, but that if the plants escaped seedling injury no root rotting occurred and the plants developed normally like the check plants. He considers *Fusarium moniliforme* and other species of the elegans section to be concerned only as secondary invading organisms. He concludes that the organisms heretofore considered as etiological in the corn root rot problem are probably able to produce seedling blight only, but that true corn root rot which occurs comparatively late in the development of corn plants, is a distinctly different disease produced by an altogether different fungus.

Johann, Holbert, and Dickson[64] reported in an abstract in Phytopathology that an undetermined species of Pythium caused considerable seedling blight of corn in Illinois and Wisconsin under conditions of comparatively low soil temperatures and high soil moistures. They found that if the infection was not severe enough to kill the seedlings, they were more or less retarded in size and vigor by the soft rot of the feeding roots.

In the review of the literature just given, it is seen that ear rot diseases, caused especially by Diplodia and Fusarium species, have been well known for more than twenty years, while the knowledge of the disease of corn roots is comparatively recent in origin. In 1918 when corn root rot first became recognized as a serious disease all over the corn belt, the most important manner of transmitting it was thought to be through planting seed corn infected with organisms as *Diplodia zeae*, *Gibberella saubinetii*, *Fusarium moniliforme*, and others. Accordingly, plant pathologists who were familiar with the problem recommended the most effective means of control to be the planting of seed corn comparatively free from fungous infection. Later it was found in Tennessee, Kentucky, and Nebraska that lightly infected seed corn selected by the approved germinator method gave no better yields than heavily infected seed, thus showing the unimportance of infected seed corn in producing corn root rot in those states. Other investigators found the organisms ordinarily present in seed corn to be responsible for seedling blights, but no direct evidence was given to show their capabilities of producing corn root rot. One type of root rot was found to occur generally when corn was grown on soils deficient in lime and available phosphate and potash. More recently results from experiments carried on in Kentucky suggest that only seedling blight diseases result from the more common seed-borne

fungi, while true corn root rot is produced by a different fungous organism that is not carried by the seed but is soil-borne.

THE OCCURRENCE OF FUNGI IN CORN GRAINS IN MISSOURI

The investigational work reported in this paper began in 1921. The work has been in progress continually since that time except for the period from December 1924 to September 1926. Some of the experiments were carried on in the laboratory, some in the greenhouses, and others in the field. The first experiments carried out were performed in the laboratory and dealt with the occurrence of fungi in seed corn grown in Missouri. At that time the importance of disease-free seed was stressed almost exclusively by investigators engaged in the root rot problem. There seemed to be some differences of opinion regarding the species of organisms in seed corn in various states so that the first problem suggested to the writer was to determine the specific organisms infesting corn in Missouri.

Seat of Infection in Kernels.—Before determining the species of fungi in corn kernels, the problem of working out a practical and satisfactory technique presented itself. After various preliminary trials of many substances for surface disinfection of corn kernels, it appeared that the best means of disinfection was to immerse the kernel momentarily (10-20 seconds) in 95% alcohol, then soak one minute in a 1:1000 mercuric chloride solution, and wash several times in sterile water before placing the kernel on agar. Whole kernels seemed rather difficult to manage in petri dishes, particularly when one attempted to bury a large part of the kernel in the agar. Consequently, an effort was made to determine the distribution of the fungus in the kernel. It was also thought that information on the distribution of the fungi in the grain might yield suggestions on the pathway of infection and methods of disinfecting corn grains. Microscopical examinations of kernels from infected ears showed fungous mycelium present almost invariably just beneath the tip cap*. In many cases, fungous mycelium could be observed in the cavity containing the embryo, which did not completely fill the cavity.

Microscopical examination, however, is slow and tedious and uncertain because of the possibility of overlooking the mycelium. The following procedure, therefore, was used; two representative kernels

*It might be well here to give a brief description of the morphology of the corn kernel. The kernel, seed, or grain as it is commonly called is a ripened fruit or caryopsis. It consists of a mature ovary with all its adnate parts. The kernel is established in a "fruit cup" formed by six fruit envelopes. According to Winton[125] this cup consists of "three glumes—a flowering glume, a palet, and another palet belonging originally to a rudimentary blossom." The cap of the kernel consists of a dried tissue, non-functioning at maturity. The inner structure of the cap consists of thick-walled cells and several vascular strands. This cap if partly cut or broken off exposes a small cavity over the dorsal extremity of the scutellum. There is also observed a brown covering adhering to the lower dorsal end of the scutellum. The cavity varies in size and the covering over the scutellum is light brown to black, according to the variety of corn.

from each of 100 ears of seed corn selected at random were cut into five pieces as shown in the accompanying diagram (Fig. 1).

The pieces were surface sterilized and washed according to method described above and placed in sterile petri dishes each containing 30cc. of potato dextrose agar. All five pieces of a single kernel were placed in a petri dish, kernel section number one in the middle and the other sections equidistant from number one and a little nearer the edge of the dish. A strong pair of tweezers was used to force each piece of kernel into the agar so that less than half of the surface was left exposed to the air.

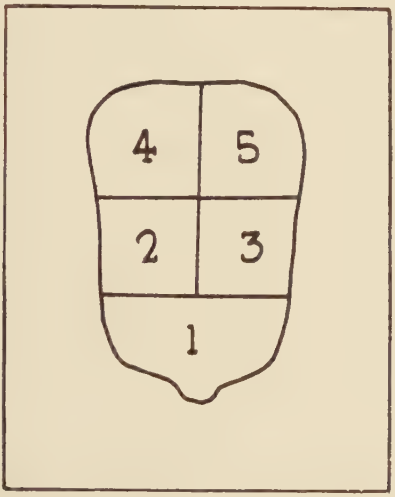

Fig. 1.—Each kernel was cut in five approximately equal pieces.

The potato dextrose agar used in this experiment and all other work in plating corn kernels was prepared in the usual way by boiling 200 grams of peeled sliced potatoes until soft, filtering through cheese cloth and adding 20 grams of agar, 10 grams of dextrose and sufficient water to make one liter.

The plates were incubated for eight days at room temperature (about 25°C.) and then examined for fungous colonies. Table 1 shows the results obtained from this experiment.

These results seem to warrant the assumption that if a corn grain is infected, the tip one-fifth invariably contains the fungus, since in no case when the tip failed to show infection did any other part of the kernel show infection.

Although these data give no light on the manner in which fungi reach the ear, there is evidence to show that the tip of the kernel is more

readily penetrated by fungi. Many kernels (31 per cent)showed infection only in the tip one-fifth; the fungi evidently did not penetrate the remainder of the kernel. Selby[100] assumed that the fungi attacking corn

TABLE 1.—NUMBER AND KIND OF ORGANISMS IN VARIOUS KERNEL SECTIONS
Legend: D, *Diplodia zeae*; F, *Fusarium spp*; C, *Cephalosporium acremonium*; A, *Aspergillus spp.*; P, *Penicillium spp.*; and R, *Rhizopus spp.*

Ear number	1	2,3	4,5	Ear number	1	2,3	4,5
1	F	0	0	51	2C	2C,P	0
2	2F	2F	0	52	F,C	3A	0
3	0	0	0	53	2F	A	0
4	2F	0	0	54	2F	3F, A	2F,A
5	2D	2D,C	D,C	55	2C	4A	C
6	F,C	D,R	0	56	2F	2A	4A
7	2F	0	0	57	2F	4A	A
8	2F	4F	0	58	2F	4F	0
9	F	C	0	59	F,D	2D,2F	2F,A,P
10	0	0	0	60	C,F	C,A	2A
11	2F	0	0	61	F,C	2D,P,A	3A
12	2F	0	0	62	C,F	0	2P
13	2F	4F	F	63	2A	4A	3A
14	2F	0	0	64	2F	2F,2A	4A
15	F,D	4D	2F	56	2F	3A	3A,P
16	2F	2F	0	66	2C	2F	F
17	2F	C,F	D	67	2F	3F,P	A
18	2F	2F	F	68	C,F	2A,2F	0
19	2F	4F	0	69	F,C	2D,A	2D,P
20	2F	3F	F	70	F,C	2F,P	D
21	2F	0	0	71	2F	2F	2F,A
22	F,C	0	0	72	2D	4D	2F,A
23	2F	4F	2F	73	2C	2C	3P
24	2F	2C	0	74	2P	4P	4P
25	F,C	0	2F	75	C,F	2C,P	3P
26	2F	0	0	76	2C	C	A
27	2F	F	2F	77	2F	3F	3P,F
28	2F	2F	0	78	2D	4D	2F,2D
29	2F	0	0	79	C,F	2F,C	2F,P
30	2F	0	0	80	2P	4P	2P
31	2F	0	0	81	0	0	0
32	2C	0	0	82	2C	0	0
33	C	0	0	83	2C	0	0
34	2C	0	0	84	2C	0	0
35	2C	0	0	85	2C	P	0
36	2C	0	0	86	2F	0	0
37	2D	4D	D	87	2F	0	0
38	2C	C	0	88	F,C	2F	0
39	2F	0	0	89	2F	3F	4F
40	2C	C	0	90	2C	0	0
41	2F	0	0	91	2F	4F	3F,P
42	2F	2F,D	0	92	2F	3D	0
43	2F	2F	0	93	F,C	3F	P
44	2F	3F,D	2F,P	94	2F	4F	2F
45	2F	2F	0	95	2D	4F	2P
46	2F	3F	0	96	2F	F,C	2C,2P
47	2F	0	0	97	2F	4F	F
48	2C	P	0	98	2F	4F	3P
49	2F	3F	0	99	C,D	3F,C	2F,D
50	2F	0	0	100	2P	4P	4P

grew up the stalk from the roots, entered the shank, and penetrated the kernels by way of the pith in the cob. That such a path of infection is followed has been proven for one organism only, *Cephalosporium acremonium*, by Reddy and Holbert[91]. The writer and others[18,42] consider the principal source of kernel infection is by way of wind-borne spores lodging in the husks at the tip or butt of the ear since corn stalks containing no organisms whatever in the internodal tissue may and usually do bear an infected ear. Manns and Adams[76] found infection to be concentrated in the tip end of kernels for they reported that when the tip cap was removed a greater number of seedlings free from severe infection were obtained. This was thought to be the result of eliminating the greater part of the internal fungous infection. It was further established that by disinfecting kernels after removal of the cap, germination unaccompanied by developing fungi in many instances was secured. But whether the fungus enters the kernel tip by way of the cob pith and cob proper after having gained entrance at either end, or whether it enters after having developed somewhat between the rows of kernels without entering the interior of the cob is a question yet to be determined.

The Prevalence of Ear Infection as Shown by a Disease Survey of Seed Corn.—During the winter of 1921-22 representative seed ears of corn from all sections of Missouri were collected. Every county agent and many farmers were asked to send in four ordinary seed ears; two with clean, white butts and sound tips, and two with black, brown, or reddish discolorations in the butt end of the cob and with discolored and slightly molded tips. Samples were received from 49 counties, including all the principal corn-growing counties in the various sections of the State. As each sample arrived the two apparently infected ears were numbered one and two. The two remaining ears, though in most cases not free from apparent infection in the writer's judgment, usually showed disease symptoms to a less marked degree, and were numbered three and four. The source of each sample is shown in Table 2.

Eight representative kernels, located spirally from butt to tip, were removed from each ear. The tip one-fifth only from each kernel was isolated because it could be manipulated easier in sterilizing and placing in the agar in the petri dish, and also because the elimination of seedling growth was decidedly advantageous. This method in no way affected the accuracy of the results since the previous experiment showed that the tip was sure to be infected if any other part of the kernel was infected. The technique followed in making the isolations was the same as described in the previous experiment. Four kernel tips were put in each petri dish. The plates were incubated 20 to 30 days at room temperature of about 25°C.

An examination of the results given in Table 2 shows that of the 192 ears tested from 49 counties, all but 17 were infected with seed-borne fungi. Corn ear samples showed 100 per cent ear infection from 33 counties, 75 per cent ear infection from 15 counties, and 50 per cent ear infection from one county. There was no evidence to indicate that corn

TABLE 2.—SOURCE AND PERCENTAGE INFECTION OF SEED CORN STUDIED IN DISEASE SURVEY

County	Number of ears received	Percentage ears infected	Percentage tested kernels infected
Andrew	3	100	33
Atchison	4	75	37
Bates	4	75	34
Buchanan	4	100	87
Butler	4	100	75
Caldwell	4	75	72
Callaway	4	100	68
Cape Girardeau	4	100	96
Carroll	4	75	50
Cass	4	100	87
Chariton	4	100	91
Clinton	4	100	53
Cole	4	75	56
Dekalb	4	100	63
Dent	4	100	81
Dunklin	4	100	66
Douglas	4	75	63
Gentry	4	75	31
Green	4	100	53
Harrison	4	75	44
Holt	4	100	16
Howard	4	100	70
Howell	4	75	50
Jackson	4	100	59
Jasper	4	100	72
Jefferson	4	50	13
Knox	4	100	85
Lafayette	4	100	63
Lewis	4	100	66
Lincoln	4	100	77
Linn	4	75	50
Marion	4	75	65
Mississippi	4	100	84
Monroe	4	100	75
McDonald	4	100	97
Morgan	4	75	19
New Madrid	4	100	63
Nodaway	4	75	48
Pemiscot	4	100	72
Perry	3	100	96
Phelps	4	100	100
Pike	3	100	67
Pulaski	4	100	69
Randolph	4	75	38
Saline	4	100	52
Shelby	3	100	59
St. Charles	4	100	72
St. Clair	3	67	41
Webster	4	100	47

from any particular section of the state was less infected than corn from other sections.

It was observed that more than one organism grew out of many kernels. Every possible combination of two of the organisms listed in Table 3 was observed from a single kernel. In some cases *Diplodia zeae* appeared first followed by *Fusarium moniliforme*, but in most cases a fusarium colony was followed with a growth of *Cephalosporium acremonium*. There was considerable difference with regard to the rapidity of growth of the three organisms mentioned. Diplodia grew out most rapidly if present and oftentimes the colony overgrew the nearest neighboring kernel tip by the time another fungus appeared from it. Likewise *Fusarium moniliforme* grew out much faster than *Cephalosporium acremonium* and a fusarium colony often reached an adjacent kernel infected with *Cephalosporium acremonium* by the time the latter had made any visible growth. There was little difficulty in identifying the fungi that grew out of one kernel because of the difference in appearance of the three organisms on the medium used. Diplodia produced abundant white cottony mycelium and in old cultures—two weeks or more—turned the agar dark to grayish black in color; *Fusarium moniliforme* made a sparser mycelial growth, pink in color, and turned the agar dark blue to purple in color; while *Cephalosporium acremonium* produced a very slow-growing, dense, light pink mycelial growth that later formed a leathery mat on the agar. In all such cases, where more than one fungus grew out of a single kernel, only the organism that appeared first was counted. Hence many of these kernels reported as being infected with *Diplodia zeae* were also infected with *Fusarium moniliforme* or *Cephalosporium acremonium* or possibly both. Many such kernels reported as being infected with Fusarium were also infected with Cephalosporium but never with Dilpodia. Kernels indicated in Table 3 infected with Cephalosporium only contained neither of the other organisms, else they would have grown out first. Table 3 shows the relative occurrence of the three species of fungi as found in Missouri seed corn grown in 1921.

The first group, or apparently infected ears, contained nearly twice as many kernels infected with Diplodia and Fusarium as the second group; but the latter group, or apparently fungus-free ears, contained many more kernels infected with the third organism, Cephalosporium. This may be explained by the fact that both Fusarium and Cephalosporium grew from many plantings, but since the latter organism invariably appeared two to ten days later than Fusarium it was not counted. Therefore, since ears one and two were so heavily infected with Fusarium few kernels were left out of which Cephalosporium alone might grow. Considering the isolations that yielded more than one fungous

outgrowth, there was no evidence to indicate that the second or best group of ears were more heavily infected with Cephalosporium than the first or more diseased group of ears.

TABLE 3.—SHOWING RELATIVE OCCURRENCE OF FUNGI FOUND IN SEED CORN

	Number of ears	Number of isolations	Percentage of isolations	Percentage of ears infected
Fusarium moniliforme				
Ears 1, 2	98	319	40.7	79.6
Ears 3, 4	94	192	25.5	57.5
Total	192	511	33.3	68.8
Cephalosporium acremonium				
Ears 1, 2	98	110	14	46.9
Ears 3, 4	94	179	23.7	59.6
Total	192	289	18.8	53.1
Diplodia zeae				
Ears 1, 2	98	93	11.9	34.7
Ears 3, 4	94	39	5.2	18.1
Total	192	132	8.6	26.6

The writer[11], in publishing a report of this experiment in the Journal of the American Society of Agronomy in 1922, referred to *Cephalosporium acremonium* as *Cephalosporium sacchari* because, as stated then, it resembled the organism tentatively given that name by Manns and Adams of Delaware. No attempt was made by the writer to name the organism, but Reddy and Holbert[91] definitely determined an apparently identical organism to be *Cephalosporium acremonium* which they described as the causal organism of the black-bundle disease of corn.

It is interesting to note here that only one culture was positively identified as *Gibberella saubinetii* from all the isolations, which totaled more than 1600. Manns and Adams[75], in making a similar survey of Delaware corn grown in the season of 1920, found that *Cephalosporium acremonium* appeared in 39.54 per cent of the isolations. *Fusarium moniliforme* in 19.92 per cent; *Diplodia zeae* in 5.69 per cent; and in addition *Gibberella saubinetii* in 5.95 per cent. It appears that Gibberella is very much less prevalent as a parasite of corn in Missouri than it is in Delaware. Other investigators give no definite figures on the prevalence of various fungi in seed corn, but Hoffer and Holbert[43] stated in their first publication on corn root, stalk, and ear rot diseases that "The harmful organisms referred to in this bulletin are species of Gibberella, Fusarium, Verticillium, Rhizopus, and Pseudomonas. In a later paper[55] they report that *Gibberella acervalis*, *Gibberella saubinetii*, Rhizopus, and Aspergillus, as well as certain bacteria may occur on diseased, weak, or immature kernels. Holbert[58] and co-workers at Illinois consider *Rhizopus spp.*, *Diplodia zeae*, *Fusarium moniliforme*, *Gibberella saubinetii*, and *Ceph-*

alosporium acremonium as the most important seed-borne pathogenes causing corn root, stalk, and ear rot diseases. Many pure culture isolations have been made in this laboratory nearly every year since the work reported above was done and there apparently has been no increase in the amount of seed infection by *Gibberella saubinetii*. The only explanation the writer is able to offer for the seeming absence of *Gibberella saubinetii* as a parasite of seed corn in Missouri, as compared with Illinois, Indiana, and Delaware, is that wheat scab is much less abundant in Missouri.

Detecting Comparatively Disease-free ears by Means of Physical Ear Characters.—It was shown in the test just described that ears with discolored butts and moldy tips were infected to a greater extent with seed-borne fungi than ears which lacked these characters. After completing this test, the writer went through the lot of ears and very carefully selected all the ears that appeared perfectly clean, healthy and sound. In this selection, in addition to selecting those ears free from conspicuous molds or unsoundness, much care was taken to avoid brown, black, red, or dark discolorations and the slightest signs of mold in the butt ends of the cobs and extreme tips of the cobs. The idea in mind was to select disease-free or nearly disease-free ears on the basis of external ear characters. How effectively this was done is illustrated in Table 4, which gives the number of fungous infections in apparently disease-free ears selected after the manner described above compared with the fungous infections in a similar number of ears selected for their diseased appearance. The data in Table 4 were compiled from the survey reported in Table 2.

It will be noted that only 19 of the 192 seed ears appeared completely clean, sound and healthy. Nine of these ears were disease-free in pure culture and only four ears that proved to be infected showed as much as 50 per cent infection; on the other hand, the ears selected for their diseased appearance generally showed 100 per cent infection. Further examinations of the ears selected as apparently disease-free revealed a very consistent difference in kernel character between the infected and non-infected ears. When kernels were pulled in the ordinary manner from the infected ears the tip cap usually broke away from the kernel and remained attached to the cob; while in ears free from infection the tip cap usually remained attached to the kernel that was pulled. This suggests that either the fungus growing in a kernel weakens or partially severs in some way the connection between the kernel proper and the tip cap or that the kernel is more securely attached to the cob. In the case of badly diseased ears many pulled kernels revealed the tip of the embryo exposed so that a part of the endosperm as well as the tip cap was left attached to the cob.

TABLE 4.—SHOWING RELATIVE INFECTIONS OF APPARENTLY SOUND AND APPARENTLY DISEASED SEED CORN

Number of isolations from eight kernels from ears selected as disease-free.

Ear Number	*Fusarium moniliforme*	*Cephalosporium acremonium*	*Diplodia zeae*
2.4	0	0	0
3.3	0	0	0
9.3	0	1	0
13.3	2	0	0
13.4	3	0	0
14.3	0	0	0
14.4	0	0	0
18.3	0	0	0
21.3	0	0	0
21.4	0	2	0
25.4	0	0	0
31.3	3	0	2
32.4	0	0	0
34.3	5	0	0
51.3	0	0	0
54.3	2	2	0
57.4	3	5	0
69.3	0	0	0
73.4	0	0	2
Total	18	10	4
Number of isolations from eight kernels from ears selected as diseased.			
1.1	1	2	2
3.1	7	0	0
4.1	0	0	8
5.1	0	1	5
5.2	6	1	1
8.1	5	0	3
8.2	4	0	4
9.1	4	0	3
9.2	4	4	0
10.1	4	0	3
16.1	1	6	1
18.1	6	1	0
24.1	8	0	0
30.1	1	7	0
31.1	5	0	2
36.1	7	0	1
39.2	8	0	0
40.1	8	0	0
51.1	3	2	2
Total	82	24	35

It will be recalled (Table 2) that in the disease survey studies seventeen ears were found to be free from internal fungous infections. Before any ear could be considered absolutely disease-free the writer felt that repeated isolations of representative kernels in pure culture should be made until a total of at least 40 kernels had been found to be fungus-free. Then one could be practically certain, though not absolutely certain, that all the kernels on such an ear were free from internal fungous infection. Accordingly, 32 more kernels from each of the ears mentioned above were isolated and grown in pure culture. Only two of the ears

were found to be disease-free according to such an arbitrary standard. It is significant to note that in testing kernels in pure culture from more than 500 ears of corn during the course of these investigations only nine disease-free ears have been found. The methods used in obtaining two disease-free ears for use in the greenhouse experiments performed in 1926 -27 illustrates how difficult it is to find such ears. From eight bushels of high grade Reid's Yellow Dent and Boone County White seed corn 14 ears were selected on the basis of certain physical ear characters described below as being apparently free from infection. Kernel isolations from these ears showed only two of them to be disease-free. Thus it appears that the percentage of disease-free ears in an ordinary unselected lot of corn is indeed very small.

As a result of the above tests and much subsequent experience in selecting nearly disease-free ears with the modified rag doll germinator, it is suggested that for all practical purposes certain physical ear characters may be substituted for the "germinator" method, recommended by Hoffer[43], Holbert[54] and others[25,71,75], in selecting comparatively disease-free seed corn. These physical ear characters were described in a circular[12] written for distribution among Missouri farmers as follows:" First, select ears that are firm and heavy, having bright, plump kernels. Avoid light ears or ears easy to twist. Second, select ears with no visible signs of molds. Avoid white, gray, pink, red or other colored molds on the ends or other part of the ear. Third, select ears with clean white, bright butts where the shank has broken from the cob. Avoid discolored butts. Fourth, select ears with clean, bright cob tips. It is not important that the tips be covered with kernels. Avoid ears with moldy or badly discolored cob tips. Fifth, avoid ears whose kernels break near the tip when pulled out."

All except the fifth of these points have been recommended by different investigators in selecting seed ears before testing on the germinator. Manns and Adams[75] have found that "Ears having smooth, properly dented kernels, somewhat flinty, make better seed and are freer from disease than the rough ears, which are usually very starchy," Valleau[116] found that "selection for the extremes of smooth ears and rough ears resulted in increases of 39.4 and 13.9 per cent in yield for the smooth type over the rough." Kiesselbach[70] found that although slender, smooth ears with horny kernels showed ten to twenty per cent greater freedom from root rot diseases than large, rough, starchy, deep-grained ears, they produced no better yields than the badly diseased ears selected by the germinator method from the same lot of corn. According to Holbert[58] and others "Disease-free or nearly disease-free ears have thick, plump, bright, clean kernels with well developed germs, intermediate to smooth indentation, and distinctly horny endosperm. Such ears are sound and

solid, have a bright rather oily appearance, and give every evidence of complete and normal maturity. Shank attachments are white, bright, and free from any discoloration due to exposure or disease. All ears that do not measure up to these standards, or as many as the seed stock will allow, should be discarded as they probably are more or less diseased."

Hoffer and Holbert[43] early in their studies of corn root rot emphasized the importance of avoiding "starchy"ears for seed which they found were more susceptible to disease and were infected to a greater extent than horny ears. Largely due to the efforts of Holbert, working at Funk Brothers Farms at Bloomington, Illinois, a strain of corn was developed and recommended to farmers as being more disease resistant than other kinds of corn. The corn was called the "Utility Type". It was a selection for disease resistance from Reid's Yellow Dent with rather slender ears and medium shallow kernels nearly smooth in indentation and very horny in composition. An exhibit of Utility Type corn was made by the University of Illinois at the National Grain and Hay Show in Chicago in 1920 and 1921 in which yield tests were given to show that in certain sections of Illinois Utility Type corn outyielded all other good strains of the more generally accepted so-called "rough" types of corn. It was suggested that corn with comparatively rough indentation was inferior in yielding qualities to the Utility Type partly because of the latter's disease resistance qualities. This disapproval on the part of Illinois of the existing types of Reid's Yellow Dent and Johnson County White corn met much opposition in the near-by state of Indiana. The Indiana Corn Growers' Association[113] in 1922 published a pamphlet showing the inferiority of Utility Type corn in Indiana. Three kinds of Utility Type corn were compared in a yield test with three high yielding strains of Indiana corn of the generally accepted types. The Indiana strains consistently outyielded the Utility Type strains in all five tests conducted in different parts of the state. The average yield of Indiana corn over Illinois corn was 5.9 bushels per acre. The pamphlet stated further "The prize-winning samples presented in this circular, which represent important Indiana types or strains of corn, have come out of fields yielding 79 bushels to 129 bushels per acre. These are highly creditable yields and Indiana corn growers may well continue to feel just pride in the type of corn for which they have been working. Furthermore, the yield test herein reported shows that Indiana types of corn have higher yielding ability than leading representatives of the so-called utility types. The criticism directed against the Indiana type of corn, all of which comes from outside the state, has been based upon theories. In like manner, only theories and inflamed statements without facts have been produced in support of the so-called Utility Type of corn." The lack of influence exerted by the Utility Type of corn on the generally accepted

standards is evident because the kernel type of the leading varieties of corn, such as Reid's Yellow Dent, Boone County White, and Johnson County White, has not changed appreciably. It is noticeable, however, that fewer practical corn breeders are holding to extreme types of rough indentation in the strains they are growing and developing.

In the writer's judgment Kiesselbach[70] offers the most reasonable explanation for the discovery by some investigators that ears with horny kernels outyield ears with very starchy kernels. He states in conclusion from his investigations under Nebraska conditions that "Whenever corn types are being grown which tend to be somewhat too large and late maturing for their environmental conditions, selection of this smooth type of ear, whether because of root rot disease considerations or otherwise, is likely to result in increased production because of the better adaption of plant type represented in this type of ear. These type considerations apply where the various types are selected from the same general variety of corn."

Dungan[26] has recently found that the so-called starchy corn contains no more total starch than horny corn, and frequently not so much. He suggests that the term "floury" be applied to corn having a large amount of soft starch. Seedlings from horny kernels are more vigorous than seedlings from floury kernels, he states, because there is often a greater food supply in the horny kernel and because the horny starch is more readily hydrolyzed to a soluble condition than the soft starch.

Recently, Jehle, Oldenberg, and Temple[63] reported yield tests in Maryland showing a relation of internal cob discoloration to yield in corn. They conclude from the results secured in ninety tests that the yield from seed corn on cobs free from internal discolorations is greater than the yield from seed corn on cobs with internal discolorations; and that the greater the internal discoloration, the smaller the yield. In determining the internal cob discolorations, they found that chopping off about two inches of the butt and tip of the ear before making the observations was more accurate than simply examining the two extremities of the cob. It was found that some cobs showed externally discolored butts but were perfectly free from discolorations at all points more than one inch from each extremity. This would indicate that the Maryland method of selecting seed ears on the basis of physical characters would secure more ears from a lot of corn than the method recommended by the writer, since in the latter case all ears with discolored butts would be thrown out. The writer questions, however, whether the possibility of securing a few more seed ears would compensate for the added inconvenience and waste involved in chopping off the buts and tips of all ears examined.

THE RELATION BETWEEN KERNEL INFECTION AND CORN ROOT ROT IN THE FIELD

The fact that Missouri seed corn grown in 1921 showed practically no infection with *Gibberella saubinetii* seemed surprising, especially in view of the fact that Hoffer and Holbert considered it at that time to be the principal organism infecting seed corn and causing root rot in Indiana and Illinois. It seemed desirable then to determine whether or not Missouri seed corn heavily infected with fungous organisms actually produced more root rot in the field than slightly infected seed. If *Gibberella saubinetii* carried in the seed was the pathogene largely responsible for corn root rot, comparatively little disease could be expected from planting Missouri seed corn.

Field Experiment in 1922.—Methods Used.—Plans were made during the early months of 1922 to compare in field experiments yields from heavily infected seed with yields from slightly infected seed. These experiments were conducted on the outlying experiment fields of the Missouri Experiment Station at Maryville, Stark City, Cuba, Warrensburg, and Kirksville. Seed corn was obtained from farmers in the vicinity of each of the experiment fields so that the seed used in planting each test would be adapted to that particular locality. About twenty times as much seed as needed for planting was obtained for each test so that there might be plenty of corn from which to select the kinds of seed needed. This corn was tested by means of the modified rag doll germinator described by Hoffer and Holbert[43]. Eight kernels were removed spirally from butt to tip of each ear and grown eight to ten days in the germinator. Only those ears having seven or all eight of the seedlings badly rotted were placed in the "diseased seed" lot. Much care was taken to avoid ears that did not give 100 per cent germination. Only those ears that showed no seedling rot on the germinator were used for "clean seed". Hereafter in this paper seed corn that shows badly rotted seedlings in the germinator will be referred to as "diseased" seed and seed corn that shows little or no rotting in the germinator will be referred to as "clean" seed. By "disease-free" seed is meant that coming from those ears from which 40 representative kernels have shown no internal infection when germinated on agar under sterile conditions. In each test the diseased and clean seed was planted in alternating replicated plots.

Table 5 shows the average yields of clean and diseased seed in five different tests. The differences are significant in only two cases, at Maryville and Warrensburg, and the writer is of the opinion that the Maryville data should be discarded because of the inaccurate manner in which the yields were determined by the man in charge of the harvesting. Some member of the Experiment Station staff had charge of harvest-

ing the corn at the other fields. This leaves only one test in which the difference in favor of clean seed was significant. However, only one test showed the diseased seed actually yielded more, and in this case the difference was not significant.

TABLE 5.—YIELDS FROM CLEAN AND DISEASED SEED IN 1922

Outlying field	Variety seed used	Average yield in bushels per acre[1]		Difference in favor of clean seed[2]
		Clean	Diseased	
Maryville	Reid's Yellow Dent	63.0±1.5	55.8±1.6	7.2±2.2
Stark City	Commercial White	40.1±3.4	41.7±2.3	−1.6±4.1
Cuba	White Pearl	8.9±0.7	8.0±0.9	0.9±1.1
Warrensburg	Boone County White	38.9±1.1	33.5±0.8	5.4±1.4
Kirksville	Reid's Yellow Dent	50.5±3.5	49.0±3.4	1.5±3.7

[1]. The probable errors were calculated by Peter's formula, thus:

$$E = \pm 0.8453 \times \frac{V}{N\sqrt{N-1}}$$

where V is the sum of the variations from the mean, and N the number of variates.

[2]. The difference between two values (mean yields) in this case is considered statistically significant when its value is 3.2 (or more) times its probable error. The probable error of a difference in two values, each having a probable error, is determined by the formula—

$$E \text{ of difference } \sqrt{E^2_a\ E^2_b}$$

where E_a is the probable error of one of the values under comparison and E_b is the probable error of the other.

The only conclusion that can be drawn from these data is that under the conditions of the experiment in 1922 there was practically no difference in yield between diseased seed corn and clean (comparatively non-diseased) seed corn. The large differences in yield usually obtained by other investigators[56,57,58,63] in comparing diseased and clean seed led the writer to believe that differences in stand in diseased and clean plots must be an important factor. Although no information concerning the comparative stands in the clean and diseased seed plots was obtained in the Missouri experiments in 1922, it was assumed that where nearly equal yields were secured approximately equal stands existed inasmuch as both lots of seed germinated 100 per cent. Therefore, plans for repetition of the experiment in 1923 were made to take into account the stand of corn in the clean and diseased seed plots.

Field Experiments in 1923.—These tests were conducted in much the same manner as those in 1922 except that in certain cases the stand of corn was determined. In addition to comparing clean and diseased seed of an adapted variety of corn, similar tests were made with unadapted Missouri corn and with Illinois seed corn. Adapted seed corn was

obtained from farmers in the vicinity of each outlying field: unadapted seed was obtained from a farmer in extreme southeast Missouri. The Illinois corn was furnished by the Illinois Experiment Station and came divided into two lots designated as disease-free and moderately diseased seed. The adapted and unadapted clean and diseased seed was selected as before from ears that germinated 100 per cent. Composite tests made on the seed received from Illinois gave 100 per cent germination for the clean seed and 90 per cent for the diseased seed.

The diseased and clean seed from each of the three lots was planted in alternating replicated plots at Maryville, Stark City, Cuba, and Warrensburg. The corn was planted in each outlying field at the usual time for planting corn in that locality. All the tests were observed in the fall just before the corn matured, but counts of plants living through to maturity were made only at Maryville and Stark City. At none of the fields was one able to distinguish with the eye any difference in appearance between diseased and clean seed plots.

Table 6 shows that the yield of Illinois diseased seed is less than the yield of clean seed in just about the same proportion that the stand is less.

TABLE 6.—YIELDS FROM ILLINOIS CLEAN AND DISEASED SEED

	Average number of plants per row	Yield in bushels per care
Maryville Experiment		
Clean seed	174	68.1±3.2
Diseased seed	164	66.4±1.4
Difference in favor of clean seed		1.7±3.5
Stark City Experiment		
Clean seed	131	45.5±2.3
Diseased seed	118	41.2±2.6
Difference in favor of clean seed		4.3±3.5

Thus at Maryville where the stand in diseased plots was 6 per cent less than in the clean plots the yields were about 3 per cent less; and at Stark City where the stand was 10 per cent less in diseased plots the yield was also 10 per cent less. It would seem from these results that the decrease in yield from planting Illinois diseased seed was not necessarily due to more corn root rot but to more seedling blight which materially reduced the stand. The difference in yield between plots planted to diseased seed and plots planted to clean seed, however, were not great enough in either case to be significant.

Counts were also made on the stand in plots planted with adapted seed, both clean and diseased, at Maryville and Stark City. Table 7 shows that the stand from clean and diseased seed was almost exactly the same in both tests, but that the diseased seed outyielded the clean seed at Maryville and the clean seed outyielded the diseased seed at Stark City.

TABLE 7.—YIELDS FROM ADAPTED SEED, CLEAN AND DISEASED

	Average number of plants per row	Yield in bushels per acre
Maryville Experiment		
Clean	153	71.7±3.6
Diseased	152	77.9±2.7
Difference in favor of clean seed		−6.2±4.5
Stark City		
Clean	104	33.7±0.8
Diseased	104	25.2±1.8
Difference in favor of clean seed		8.5±1.9

On account of the high probable error, the differences secured from clean and diseased seed at Maryville cannot be considered significant. The differences at Stark City, however, are significant and may be explained on the basis that although the diseased seed produced no more seedling blight than the clean seed, it did exert a harmful effect on the yield from resultant plants either due to after effects of partial seedling blight or to root and stalk rot.

Another test with clean and diseased Illinois seed was made at Columbia where more careful methods could be used in conducting the test, and where more opportunity could be had for frequent observations. Nine alternate plots, eight hills by twelve hills, were planted to Illinois clean and diseased seed, respectively, on the ninth of May. The accompanying diagram shows the plan of the experiment. The seed was dropped by hand three grains per hill and covered with a hoe. After the corn had been plowed twice the plots were thinned to two stalks per hill on the average. This was done in order to obtain equal and nearly perfect stands of clean and diseased seed. Where there was a missing hill, three stalks were left in the hills on either side to make up for it. Just before husking time the number of plants that came through the whole season was determined for each plot as shown in Table 8. The outside row on all sides of each plot was thrown out for the border so that each harvested plot was six hills by ten hills. These plots were situated on quite uniform soil and all appeared identically alike until late in August when a root rot spot appeared in Plot 38B. It was estimated that about one-fifth of the ears in this plot were rotted by saprophytic molds when the plants went down prematurely with badly rotted root systems.

TABLE 8.—YIELDS FROM APPROXIMATELY EQUAL STANDS OF ILLINOIS CLEAN AND DISEASED SEED AT COLUMBIA

	Number of plots	Average number plants per plot	Yield bushels per acre
Clean seed	4	103	45.9±1.7
Diseased seed	5	109	47.1±0.9
Difference in favor of clean seed			−1.2±1.9

An examination of Table 8 shows that under the conditions of the experiment there was no difference in yield between clean and diseased Illinois seed corn. The probable error of the difference in mean yield of the two series of plots is greater than the difference of 1.2 bushels. The lower average yield of the clean seed plots is to be expected on account of a slightly less stand. Although the stands were perfect after the second cultivation when all plots were thinned to 120 plants, a few more plants were destroyed during subsequent cultivations and by other causes in the clean seed plots than in the others. Furthermore, the yield in plot 38B was low compared with the adjacent plots presumably because of damage to many ears that fell to the ground when a part of the plants went down with root rot. The results from this test seem to confirm the suggestion that the superiority of Illinois clean seed over diseased seed at Maryville and Stark City was because the clean seed gave a better stand and not because less corn root rot resulted.

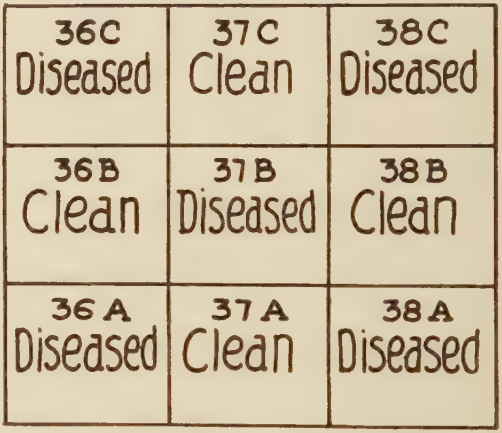

Fig. 2.—Showing planting plan of experiment with Illinois seed at Columbia in 1923.

All of the yield tests from the outlying fields are brought together and summarized in Table 9.

TABLE 9.—SUMMARY OF YIELD TESTS WITH CLEAN AND DISEASED SEED ON OUTLYING FIELDS IN 1923

Field	Clean seed, bu. per acre	Diseased seed, bu. per acre	Difference in favor of clean seed
Illinois corn			
Maryville	68.1±3.2	66.4±1.4	1.7±3.5
Stark City	45.5±2.3	41.2±2.6	4.3±3.5
Cuba	7.4±0.8	7.4±0.3	0.0±0.9
Warrensburg	12.7±1.9	10.1±0.4	2.6±1.9
Adapted Corn			
Maryville	71.7±3.6	77.9±2.7	−8.2±4.5
Stark City	33.7±0.8	25.2±1.8	8.5±1.8
Cuba	10.3±1.3	6.9±1.3	3.4±1.8
Warrensburg	37.7±1.1	39.6±1.3	−1.9±1.7
Unadapted Corn			
Maryville	70.0±0.7	61.9±1.7	8.1±1.8
Stark City	26.6±1.7	25.4±1.5	1.2±2.3
Cuba	3.8±0.3	3.6±0.4	0.2±0.5
Warrensburg	36.5±1.2	40.2±3.5	−3.7±3.6

Again the results are inconclusive. Only two of the twelve tests showed a significant difference, both of them, however, being in favor of clean seed. At Maryville the adapted diseased seed gave an 8.2 bushel increase over the adapted clean seed, but the probable error was too large to consider the difference significant. Two other tests gave a small increase in yield from diseased seed, while the remaining seven tests gave slight increases in yield from the clean seed.

Field Experiments in 1924.—Tests were conducted in 1924 at Cuba, Stark City, and at Columbia using adapted seed. In addition to plots planted to clean and diseased seed, another series of plots seeded to diseased seed at a higher rate was added and all three replicated. This latter series of plots planted to diseased seed was planted at the rate of three grains per hill as compared with two grains per hill for the other plots so as to insure at least as good a stand as in the clean seed plots. This plan was followed to test further the suggestion obtained from the 1923 results that the yields of ordinary diseased seed is roughly proportional to the stand. All plots were planted by hand and the seed immediately covered with a hoe. The plots were made smaller and more numerous in order to increase the accuracy of the results. Still another series of adjacent plots was similarly planted with "untested" seed. This seed was selected at random from each of the three lots of seed out of which the clean and diseased seed for each test was selected. As the seed corn for each test was uncrated sufficient ears to supply the "untested" seed were removed first and the remainder tested to secure clean and diseased ears. Figure 3 (page 34) shows the planting of one of the tests at Stark City which is similar to the planting plans used in the other tests.

The results from the two tests at Stark City are given in Tables 10 and 11. Both tests show that a poorer stand and a poorer yield was secured from diseased seed planted at the same rate as clean seed; but that when the diseased seed was planted thicker so that an equal or better stand was secured compared with clean seed, the yields were equal or better. In Block B it is seen that an 11 per cent reduction in stand in the plots planted to diseased seed compared with plots planted to clean seed resulted in a 17 per cent reduction in yield, while the plots seeded three-rate to diseased seed had a 31 per cent greater stand and produced only a 3 per cent increase in yield. In Block G a 20 per cent reduction in stand from diseased seed gave a decreased yield of 13 per cent, while a 16 per cent increase in stand from diseased seed planted three-rate gave only a 6 per cent increase in yield. That plots planted three-rate to diseased seed did not yield more than the plots planted to clean seed in proportion to their stand is to be expected because the optimum rate of planting corn on this soil has been found by experiment to be two-rate.

1A	2A	3A	4A	5A	6A	7A
		C	D	D3	C	D
1B	2B	3B	4B	5B	6B	7B
		D3	C	D	D	C
1C	2C	3C	4C	5C	6C	7C
		D	D3	C	C	D
1D	2D	3D	4D	5D	6D	7D
		C	D	D3	D	C
1E	2E	3E	4E	5E	6E	7E
		D3	C	D	C	D
1F	2F	3D	4F	5F	6F	7F
		D	D3	C	D	C
1G	2G	3G	4G	5G	6G	7G
		C	D	D3	C	D
1H	2H	3H	4H	5H	6H	7H
		D3	C	D	D	C
1I	2I	3I	4I	5I	6I	7I
		D	D3	C	C	D
1J	2J	3J	4J	5J	6J	7J
		C	D	D3	D	C

Fig. 3.—Showing general plan of field Experiments in 1924. Plots in each series are numbered A to J. Legend: C, clean seed; D, diseased seed; D3, diseased seed planted three-rate.

On such soil a comparatively slight increase in optimum stand gives no difference in yield, but a large increase to twice or more optimum stand actually decreases the yield. Thus we may assume that the plots planted to clean seed which had 134 plants per plot in one case and 121.1 in the other, had just about the optimum stand for that season.

At Cuba the plots planted to clean seed with 10 per cent greater stand outyielded the plots planted to diseased seed by 11 per cent, but the plots seeded three-rate to diseased corn yielded less than the plots

planted to diseased seed, although the stand was 57 per cent greater. On the basis of the Stark City tests we would expect these plots planted three-rate to diseased seed to outyield the plots planted to diseased seed and even equal or better the yield from plots planted to clean seed. However, close scrutiny of Table 12 will reveal a marked difference in the productivity of the soil at Cuba compared with that at Stark City. Here only in the most favorable years, when corn yields as much as 20

TABLE 10.—YIELDS FROM CLEAN, DISEASED, AND UNTESTED SEED ON BLOCK B AT STARK CITY, 1924

Kind of seed	Number of plots	Average number of plants per plot	Yield in bushels per acre
Adapted Commercial White Seed Corn			
Untested	20	106	45.0±1.1
Clean	10	121.1	37.3±1.3
Diseased	10	108	31.3±1.6
Diseased (3-rate)	10	158.8	38.3±1.0
Difference in favor of diseased seed 3-rate compared with diseased seed			7.0±1.9
Unadapted St. Charles White seed corn			
Clean	10	131.9	38.2±1.2
Diseased	10	123.3	34.2±1.3
Difference in favor of clean seed			4.0±1.8

TABLE 11.—YIELDS FROM CLEAN, DISEASED, AND UNTESTED SEED ON BLOCK G AT STARK CITY, 1924

Kind of seed	Number of plots	Average number of plants per plot	Yield in bushels per acre
Adapted Commercial White seed corn			
Untested	20	71.4	21.6±1.3
Clean	10	134	26.1±1.1
Diseased	10	107	22.7±1.3
Diseased (3 rate)	10	156	27.7±1.2
Difference in favor of diseased seed 3-rate compared with diseased seed.			5.0±1.4
Unadapted St. Charles White seed corn			
Clean	10	125	34.8±1.7
Diseased	10	108	31.3±1.9
Difference in favor or clean seed			3.5±2.7

TABLE 12.—YIELDS FROM CLEAN, DISEASED, AND UNTESTED SEED AT CUBA, 1924.

Kind of seed	Number of plots	Average number of plants	Number of ears	Yield in bushe s per acre
Adapted White Pearl seed corn				
Untested	20	43.3	46.5	17.0±0.8
Clean	10	108.8	110.8	19.5±0.9
Diseased	10	99.4	43.5	17.4±0.9
Diseased (3-rate)	10	122.8	40.0	16.0±1.0
Unadapted St. Charles White seed corn				
Clean	10	102.3	55.3	22.1±0.7
Diseased	10	95.9	55.8	22.3±0.9

to 25 bushels per acre, is two-rate considered the optimum for planting corn. Ordinarily, as in 1922 and 1923, the yield of corn on this field is much lower than 20 bushels per acre. Hence any appreciable increase in number of corn plants per unit area above that secured from planting two-rate may be expected to reduce materially the yield. A comparison of the number of ears per plot in the plots planted two-rate and three-rate to diseased seed further confirms the opinion that too many plants in the plots seeded three-rate reduced the yield just as effectively as too few plants might have done. In plots planted three-rate to diseased seed there was an average of 122.8 ears per plot as compared with 99.4 ears per plot planted two-rate. Though more numerous these ears were much smaller and their total weight was 8 per cent less than the 99.4 ears per plot from the plots planted two-rate to diseased seed. Apparently there was not enough available nutrients in the soil to produce more plants with well developed ears than were produced on the plots planted two-rate to clean seed. The increase in yield and number of ears from the plots planted to clean seed was in proportion to the increase in stand over the plots planted two-rate to diseased seed.

In comparing the yields from clean and diseased seed selected from a lot of St. Charles White seed, it was found that approximately equal yields were secured from an 11 per cent less stand and 7 per cent fewer ears in the plots planted to diseased seed. This variety is considerably larger in both plants and ears than the White Pearl variety; hence a stand of 106 plants per plot might well be considered more than optimum stand if 108.8 plants per plot is the optimum stand for White Pearl corn on the plots planted to clean seed.

The data secured in comparing the yields from "unselected", clean, and diseased seed planted at two different rates at Columbia are given in Table 13.

TABLE 13.—YIELDS FROM CLEAN, DISEASED, AND UNSELECTED SEED AT COLUMBIA

Kind of seed	Number of plots	Average number of plants per plot	Yield in bushels per acre
Untested	4	48	28.7±1.3
Clean	4	69.5	30.6±1.3
Diseased	4	42	23.1±1.7
Diseased (3-rate)	4	63.8	27.3±1.6
Difference in favor of diseased seed (3-rate) compared with diseased seed.			4.2±2.3

The plots planted to clean seed gave the highest average yield though not sufficiently greater than the yields from the plots planted three-rate to diseased seed and the plots planted to unselected seed to be statistically significant. Because there were only four replicated plots

planted to each kind of seed and also because of the comparatively great variation in the plots of each series as shown by the high probable errors of the means, much less weight should be attached to any conclusions that might be drawn from the results than from the results of the similar tests at Stark City and at Cuba. There is, however, nothing to indicate that the results from this test are contradictory to the more reliable results mentioned above: they actually confirm the results at Cuba and Stark City. This is the only instance in which the stand in the plots planted to diseased seed at a 50 per cent higher rate than in the plots planted to clean seed is actually less than in the latter plots. This example may be used to show the destructive effects of seedling blight as a result of planting badly infected seed corn under certain conditions.

The results secured during the three-year period from 1922 to 1924, inclusive, do not show convincingly that the amount of corn root rot as evidenced by reduction in yield is invariably and to a high degree correlated with seed infection. Although such a correlation was found by Hoffer[43] and Holbert[58] for Indiana and Illinois conditions, investigators in other states adjacent to or near to Missouri have not found this to be the case, and their results agree with those reported in this investigation.

The writer holds that in the light of the evidence already produced, seed infection in Missouri seed corn is responsible for reduced stands caused by seedling blight and is not the cause of corn root rot that appears later in the season with consequent reduction in yield. That in many tests there was no reduction even in stand from planting diseased seed suggests a possible relation to the absence of infection with *Gibberella saubinetii*. It will be recalled that this organism was considered by Hoffer and Holbert[43] to be the most important pathogene in connection with the corn root, stalk, and ear rot diseases, especially in Indiana and Illinois Moreover, this suggestion is further emphasized when it is stated that the investigators[117,80,70] in neither Kentucky, Nebraska, nor Kansas found *Gibberella saubinetii* present to any appreciable extent in the seed corn of their respective states.

SEED TREATMENT STUDIES

Seed treatment experiments were carried on at the same time the investigations were being conducted with clean and diseased seed. These experiments, which may be considered preliminary in nature, were begun with the idea of developing some practical treatment for infected seed corn that would eliminate entirely, or in part, the ill effects of seed-borne fungi. At the outset, it was found that an ordinary disinfectant, such as mercuric chloride, did not kill the fungus in the seed in most cases, but simply retarded its development when the kernel was plated aseptically on agar. This suggested that certain seed treatments might retard the

fungus sufficiently to permit seedling growth to take place normally and thus allow the resultant plant to overcome more easily the ill effect of seedling blight and its after effects.

Experiment 1.—An ear with all the kernels apparently moderately infected with *Fusarium moniliforme* was used in this experiment. Previous tests in the modified rag doll germinator showed that about a fifth of the kernels showed seedling rot after eight days. The table type of germinator described by Holbert and Hoffer[55] was used in this work because it was thought that all germinating kernels would be under more nearly identical conditions than if the modified rag doll germinator were employed. The table germinator used was six feet long by two and one-half feet wide and constructed in the form of a shallow bed four inches deep. The bottom of the bed was covered with a heavy wire screen. A layer of sawdust three and one-half inches thick was put in the bed and covered with a layer of finely ground limestone one-half inch thick. The seed was germinated between two steam sterilized muslin cloths which in turn were covered with a burlap cloth.

TABLE 14.—EFFECT OF CERTAIN SEED TREATMENTS ON DEVELOPMENT OF SEEDLING ROT IN GERMINATOR

Treatment	Dead kernels	Uninfected seedlings	Infected seedlings
None	0	63	17
Acetone and mercuric chloride	0	75	5
Alcohol and mercuric chloride	0	78	2

A total of 240 kernels were removed from the ear, mixed, and divided into three lots of 80 kernels each. One lot was soaked in acetone for one minute and then in 1:1000 mercuric chloride solution for one hour. Another lot was treated similarly with alcohol for one minute and mercuric chloride for one hour. The third lot was left untreated. The kernels were placed on the germinator in rows three inches apart both ways and germinated at 28-29°C. for ten days.

The data presented in Table 14 show clearly that the alcohol and mercuric chloride treatment greatly reduces the percentage of seedling rot on the germinator.

Experiment 2.—Another experiment was designed to determine the effectiveness of longer treatments with alcohol and mercuric chloride. A comparison was made between untreated grains and grains soaked in mercuric chloride for one- and two-hour periods. This test was similar to Experiment 1 except that more severely infected seed was used. Seed was selected from an ear heavily infected with both *Diplodia zeae* and *Fusarium moniliforme*. Fifty kernels were used in each

lot and the seedlings were germinated for nine days before observations were made.

The results given in Table 15 show practically no difference in the effectiveness of the one- and two-hour treatments. Probably because the disinfectant penetrated the kernels more deeply and killed the embryo in some cases, more dead kernels resulted from the longer treatment than from the one-hour treatment.

TABLE 15.—EFFECTS ON SEEDLING ROT FROM TREATING DISEASED SEED FOR DIFFERENT PERIODS OF TIME WITH MERCURIC CHLORIDE

Treatment	Dead kernels	Uninfected seedlings	Infected seedlings
None	20	19	11
Alcohol and mercuric chloride, one hour	14	34	2
Alcohol and mercuric chloride, two hours	17	33	0

As a result of these preliminary trials, it was decided to test under field conditions the effectiveness of the one-hour alcohol and mercuric chloride treatment in preventing seedling rot from infected seed.

Experiment 3.—Before describing the field experiments, however, it is proper to mention here the effect of long and short periods of disinfection and of temperature on the outgrowth of the organisms from an infected kernel tip placed on agar. In this test 48 kernels were used that came from a severely infected ear, all the tested kernels of which produced seedling rot on the germinator and showed in potato dextrose agar culture plates internal infection with *Diplodia zeae*, *Fusarium moniliforme* or both. None of the kernels were dead, however. The tips of the kernels were removed and the remainder discarded. Half of the kernel tips were immersed for one minute in 95 per cent alcohol and then soaked one hour in mercuric chloride, and the remainder were soaked in water for one hour. The tips were then rinsed with distilled water and dried at room temperature for one week. After the usual method of surface disinfection two potato dextrose agar plates with four kernel tips each of the mercuric chloride treated seed and two similar plates of the water treated seed were incubated at each of three different temperatures, 30°C, 25°C, and 12°C. The latter temperature was secured in an icebox which varied from 10°C to 14°C during the course of the experiment.

Infected corn kernels soaked one hour in mercuric chloride still retain the fungus in a viable form as Table 16 clearly shows. But the treatment retards the fungus in growing out of the kernel into the agar; for about three days at temperatures of 25°C to 30°C and for eight days at a temperature of 12°C. The kernels soaked for one hour and disinfected with mercuric chloride for one minute gave 100 per cent infection at all three temperatures, showing clearly that the temperature limits

for the growth of *Diplodia zeae* and *Fusarium moniliforme* are above 30°C and below 12°C. At the end of ten days, in the case of the plates containing kernels treated with mercuric chloride, the fungous colonies that appeared first had overgrown the other kernels so that one could not be sure whether or not these kernels were sterile from the treatment received. Subsequent tests, however, make the writer feel certain that the kernels were not sterile, but simply became overgrown before the fungus had time to grow out into the medium. The growth was so slow in the icebox that the plates containing kernels treated with mercuric chloride

TABLE 16.—EFFECTS OF TEMPERATURE AND SEED TREATMENT ON DEVELOPMENT OF FUNGI FROM INFECTED KERNELS

Legend: D, *Diplodia zeae;* F, *Fusarium moniliforme;* and ctn, contamination

Kind of seed	Temperature	Number and kind of colonies after:			
		Five days	Seven days	Ten days	Thirty days
Treated	30°C	1D, 1F	1D, 1F	2D, 2F	------
Untreated	30°C	7F, 1Ctn.	7F, 1Ctn.	7F, 1Ctn.	
Treated	25°C	1D	3D, 3F	3D, 3F	--------
Untreated	25°C	4D, 2F	5D, 3F	5D, 3F	----
Treated	12°C	0	0	1D, 1F	1D, 4F
Untreated	12°C	0	7F	8F	8F

for one hour did not become overgrown for 30 days, at which time three of the eight kernels had not yet produced any visible fungous growth.

It may be significant that only one Diplodia colony appeared in the 16 kernels incubated at 12°C. At the other temperatures *Diplodia zeae* grew just as often as *Fusarium moniliforme* from the infected kernels. Holbert[58] found that Diplodia infected seed yielded 46.7 per cent as much as good seed when both were planted early but the yield of the diseased seed decreased to 20 per cent of the yield from good seed when planted late in the season.

Experiment 4.—This experiment was designed to determine the effect of seed treatment on the occurrence of corn root rot in the field. Corn grown in 1920 was gathered from various sources in Missouri and ten representative kernels from each ear were germinated eight days on the table germinator. Certain ears were designated as being heavily infected, moderately infected, and slightly infected according to whether the seedlings showed a high, moderate, or slight per cent, respectively, of seedling rot while on the germinator. Six rows 400 feet long with hills three feet apart were planted to each of these three groups of seed, but the seed in three rows of each group was first disinfected by immersing momentarily in alcohol and then in a 1:1000 mercuric chloride solution for one hour.

No notes were taken on this test until September when most of the plants were just beginning to lose their natural green color and dry

up. At this time each plant was inspected for symptoms of root and stalk rot. Plants that had fallen to the ground or that were leaning at an angle of 45 degrees or more and when pulled gently, exhibited a badly diseased and rotted root system were considered to be affected with root and stalk rot.

TABLE 17.—EFFECTS FROM SEED TREATMENT ON DEVELOPMENT OF ROOT AND STALK ROT IN THE FIELD

Type of infection	Percentage of diseased plants per row:	
	Treated seed	Untreated seed
Heavy	15.5	27.4
Moderate	12.5	16.4
Light	8.9	14.4

An examination of Table 17 shows that more diseased plants resulted from planting more heavily infected seed and that disinfection of the seed as described above materially reduces the amount of root and stalk rot in the field. Unfortunately the yields from the different lots of treated and untreated seed were not determined. These results, obtained in 1921, were considered suggestive enough to justify a similar and more extensive experiment in 1922.

Experiment 5.—This experiment was planned to compare yields from disinfected diseased seed corn with yields from similar seed untreated. The seed used was secured from ears that had been found in previous tests with both the agar plate isolation and the table germinator methods to have 100 per cent kernel infection with *Fusarium moniliforme* or *Diplodia zeae* or both. This seed germinated 100 per cent. Part of the seed was "treated" by disinfecting with alcohol and mercuric chloride for one hour. The "untreated" seed, or check, was soaked in water for one hour. The treated seed was rinsed in distilled water and both lots were allowed to dry quickly. The corn was planted one week later on May 5, 1922 on Block B of the Station field. There were five replicated plots each containing three rows of treated seed and three rows of untreated seed. Three grains were dropped in hills a little less than three feet apart in rows 132 feet long.

From the first the treated seed did not appear as vigorous as the untreated seed. It was easily noticeable that the untreated seed came up more quickly and more uniformly, the plants grew off better and stayed a little ahead of those from the treated seed all summer long. When the corn was about eight inches high the hills were thinned to two stalks per hill. No counts were made at that time, but it was quite evident that there were fewer plants from the treated seed than from the untreated.

Aside from the slight tendency of the plants from treated seed to be stunted, all the corn plants developed normally with no evidence of

disease until about August 1st. A heavy wind without rain came on July 29 and another with rain came on August 6. Both these winds blew down many stalks, apparently all with greatly weakened root systems. Most of the plants that blew over did not have broken stalks, the rotted roots on the windward side simply broke off within three or four inches of the top of the ground and were left exposed as the plants fell to the ground. The total down stalks, some of which were already dead, were counted on August 9th.

TABLE 18.—SHOWING EFFECTS OF DISINFECTING DISEASED SEED WITH MERCURIC CHLORIDE

Kind of seed	Total down stalks	Average per row	Total dead stalks	Average per row
Treated	387	25.8	56	3.7
Untreated	351	23.4	64.1	4.3

It may be seen in Table 18 that more down stalks occurred in the treated seed plots, but a few less of these stalks were dead than in the check plots. Though the differences are not great, there is certainly nothing in the results to indicate that treatment of diseased seed with mercuric chloride lessened the development of corn root rot in the field.

Another observation that later proved to be quite significant was made at the time the diseased stalks were counted. There was an oval area spreading across most of the plots in which all of the plants went down and most of them died soon after. The root systems of many of these plants were badly rotted. It was also noticed that a majority of these plants bore small ears or no ears at all. This area was about 80 feet east and west by 50 feet north and south and had fairly distinct boundaries. The plots in this experiment ran north and south across the spot in which all the plants blew down. Examinations of soil in the spot revealed no difference from that outside where the corn plants had apparently healthy root systems. Examinations were made of the soil texture, structure, organic matter content, depth of surface soil, depth and character of sub-surface soil, character of subsoil to the depth of four feet, and acidity. With the brom-cresol purple indicator the hydrogen-ion concentration of a composite sample of soil in the infected area was p_H 6.4 while a similar sample outside the area where the corn plants were healthy gave a p_H of 6.0. This difference did not appear to be significant.

Table 19 gives the yield of treated and untreated seed in each plot. The higher yields in Plot 1 may be accounted for by the fact that it was just outside the spot to which attention was directed above. The inferior yields from diseased seed disinfected with alcohol and mercuric

chloride permit no doubt regarding the harmful effects of the treatment in 1922.

The value of the mercuric chloride treatment as a farm practice was tested on two fields of corn grown for silage by the Dairy Department of the University. One-half bushel of disinfected seed was planted in each field from one planter box of a two-row planter. In this way two rows of disinfected seed alternated with two rows of untreated seed. Counts made just before the first cultivation showed that seed treatment had reduced the stand 16 per cent in both fields. This result, together with the results from the yield test mentioned above, led to the conclusion that mercuric chloride as used by the writer as a disinfectant for diseased corn seed is a harmful agent rather than an aid in controlling corn root rot and seedling blight.

TABLE 19.—YIELDS FROM TREATED AND UNTREATED DISEASED SEED

Plot	Untreated seed	Treated seed	Difference in favor of untreated seed
1	52.5	38.3	14.2
2	32.6	26.5	6.1
3	30.2	28.5	1.7
4	29.5	25.1	4.4
5	30.2	26.6	3.6
Average	35.0 ± 3.0	29.0 ± 1.6	6.0 ± 3.4*

*Although the 6 bushel difference in favor of untreated seed is not significant with respect to its probable error of 3.4 bushels, the writer deems it justifiable to exclude plot 1 and compare yields from treated and untreated seed in plots 2, 3, 4, and 5. When yields from treated and untreated seed in these plots are compared the difference in favor of untreated seed becomes 3.9 ∓ 0.2 bushels, a highly significant result, statistically.

Valleau's[118] results were apparently very similar to those mentioned above, for all his attempts to control corn root rot by seed treatments gave negative results. He treated the seed with many other substances besides mercuric chloride including hot water, formalin, copper sulphate lime-sulphur, and others. Practically as much injury resulted to seedlings grown in sand following these treatments as to seedlings from untreated seed. He further stated that field results also indicated that the methods tried gave no promise of control. Mercuric chloride was also used by Reddy and Holbert[93] in 1923 for disinfecting diseased seed corn. They found that in general there was a decrease in the resulting field stands and yields from seed treated with mercuric chloride, 1:1000 for 30 or 60 minutes. They found, however, that chlorophol, semesan, and corona 620 when used as treatments for three, four, or five hours proved beneficial to stands and yields of nearly disease-free composites as well as Diplodia infected composites. The data were not given on which this statement was based. Similar experiments were repeated by Reddy,

Holbert and Erwin[92] in 1925 with diseased sweet corn seed. Results from 84 tests were reported in which different organic mercury substances and water were used to treat different lots of seed. Thirty tests gave negative results and 53 positive; however, only seven of the latter were considered statistically significant. Of these seven tests six were tests in which Diplodia infected seed was treated with semesan, semesan dust, uspulun, and corona 620. No conclusions were drawn by the authors, but apparently treatments with organic mercury compounds were effective only in increasing yields from sweet corn seed infected with Diplodia and not from seed infected with Gibberella and other organisms.

STUDY OF AN INFECTED AREA OF SOIL ON THE MISSOURI EXPERIMENT STATION FIELD

Mention has already been made of an area in Block B of the Station field in which all the corn plants went down with badly rotted root systems in 1922. This suggested very plainly that if the corn root rot was caused by an organism it must be soil-borne in this case. Although no notes were taken on this infected area in 1921, the writer remembers with certainty that a large per cent of the plants were down in parts of the same area in 1921 when the preliminary seed treatment field experiment mentioned above was planted on this part of Block B. The spot in 1922 was located approximately in the center of the west end of Block B, a plot of ground about 130 feet square that has been handled differently from any other part of the whole field. Figure 4 shows the approximate boundaries of the spot in 1922 and in 1923 as determined by down corn plants.

Previous to 1921 this plot had been seeded to sorghum in 1919 and in 1920, to corn in 1917 and in 1918, and to wheat in 1916. The plot slopes uniformly from southeast to northwest with a gradient of 3 per cent. There is evidence to show that approximately one foot of surface soil has in recent years been removed from the southeast corner and largely deposited in the northwest corner. The infected area, however, does not reach either of these regions.

In 1923 twenty rows of corn, each with seed from different sources, were planted north and south across the spot, the long dimension of which extended east and west. Some knowledge of the comparative resistance to root rot of different strains and varieties of corn was expected to be obtained from such a test. The kind and source of seed corn planted in each row is shown in Table 20.

All of the plants went down in the infected area with the exception of those in row 20. Only two adjacent hills with two plants each went down with rotted roots in this row which was thought to have at least

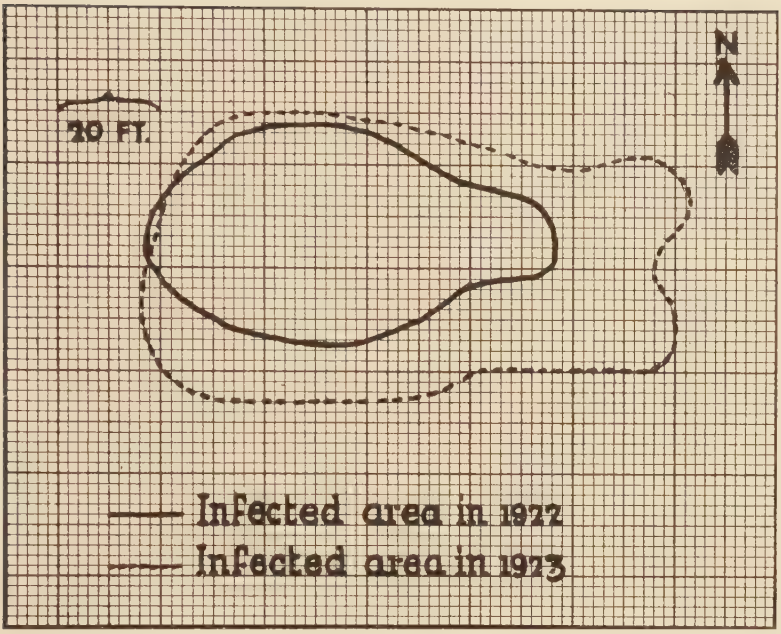

Fig. 4.—Showing approximate outline of infected area in Missouri Experiment station field in 1922 and 1923.

TABLE 20.—SHOWING KIND AND SOURCE OF SEED CORN PLANTED IN EACH OF 20 ROWS THROUGH INFECTED AREA IN 1923

1.	Bad ear	Furnished by Illinois Experiment Station.
2.	Good ear	Furnished by Ilinois Experiment Station
3.	Ear 40.1	Disease-free* ears grown in 1921 from disease-free St. Charles Yellow ear No. 2.4.
4.	Ear 40.6	Same as Ear 40.1.
5.	Ear 2.4	Disease-free St. Charles Yellow ear.
6.	Ear 3.3	Clean ear of Reid's Yellow Dent from Bates county.
7.	Ear 51.3	Clean ear of Reid's Yellow Dent from Randolph county.
8.	Ear 14.4	Clean ear of Reid's Yellow Dent from Cole county.
9.	Ear 69.3	Clean ear of Commercial White from Howell county.
10.		St. Charles Yellow diseased composite.
11.	Ear 18.3	Clean ear of Commercial White from Greene county.
12.	Ear 32.4	Clean ear of Reid's Yellow Dent from Linn county.
13.		Illinois Champion White Pearl diseased composite.
14.		Illinois Champion White Pearl nearly disease-free composite.
15.		St. Charles Yellow nearly disease-free composite from Boone county.
16.		St. Charles Yellow diseased composite from Boone county.
17.	Ear 40.3	Same as Ear 40.1.
18.	Ear 40.2	Same as Ear 40.1.
19.	Ear 40.5	Same as Ear 40.1.
20.		Clean seed composite of Boone County White from J. R. Shelton in Johnson county.

*A disease-free ear is one from which at least 40 representative kernels have been removed and when grown aseptically on agar found to be free from internal fungous infection.

five hills within the boundaries of the spot as it occurred in 1922. This row, however, was calculated to be quite near the boundary of the spot, but might easily have been outside it on account of the inexact measurements made of the spot in 1922. The spot evidently increased in size somewhat in 1923 as shown in Figure 4. The boundaries were not as distinct as in 1922; that is, the plants in hills near the border were often neither all down nor all erect. Although scattered plants all over the whole plot showed typical root rot symptoms, the disease seemed to be much more virulent in a more or less definite area. The fact that plants from disease-free seed as well as from diseased seed were equally affected in the spot still further confirmed the idea that some organism, agent, or condition in the soil was responsible for the corn root rot disease.

The work of Hoffer and Carr[46], published in 1923, in which they stated that the most severe cases of root rots had been found in soils notable because of their deficiencies in lime and available phosphates, suggested to the writer to test the effects of lime and phosphate soil treatments on the appearance of corn root rot in the infected area in 1924. The field was plowed late in the fall of 1923 and during the following spring the soil was prepared in the usual way for planting corn. Two-row plots running north and south across the infected area were laid off and given the different treatments shown in Table 21.

TABLE 21.—SHOWING PLAN OF SEEDING AND SOIL TREATMENTS IN INFECTED AREA IN 1923

Plot	Rows	Seed	Soil treatment
1	2	Badly diseased	None
2	2	Clean	None
3	2	Clean	2500 lbs. hydrated lime per A.
4	2	Clean	2500 lbs. hydrated lime plus 200 lbs. 16% phosphate per A.
5	2	Clean	200 lbs 16% phosphate per A.
6	2	Clean	None
7	2	Clean	None
8	2	Clean	2500 lbs. hydrated lime per A.
9	2	Clean	2500 lbs. hydrated lime plus 200 lbs. 16% phosphate per A.
10	2	Clean	200 lbs. 16% phosphate per A.
11	2	Clean	None
12	2	Clean	None
13	2	Clean	None
14	2	Badly diseased	None

The lime and phosphate were applied on the surface by hand, care being taken not to let the treatments overlap the adjoining plots. Immediately after application the material was raked in the top two or three inches of soil by hand. After a period of ten days the corn was planted by hand at the rate of two grains per hill on June 6, 1924. The soil was warm and the corn came up quickly and uniformly. From the

first the plants in the plots treated with phosphate were more vigorous, had a darker green color, and grew a little faster than the other plants. No differences were apparent in the plants of other plots.

An observation made on July 26 just after the plants had begun to tassel showed all plots in all parts of the field to be equally thrifty and growing well. The corn on the phosphated plots, however, still appeared more vigorous and a little larger than the remainder. Unfortunately, the writer changed his residence at this time and was not able to make further observations as the corn matured, the time at which the root rot had appeared in previous seasons. A member of the Experiment Station staff reported that by the first of October practically half of the plants were down in all the plots running through the infested area as mapped in 1923. No counts were made, but the report stated that there was no evidence of less root rot where the phosphate or lime, or both, had been applied. This evidence, though meager, indicates that root rot is due to some soil-borne organism or to some soil condition other than the lack of available lime or phosphate or both in the soil. Also, the fact that plants from diseased seed and from clean seed went down alike seemed to prove, in this case at least, that soil infection or a soil condition is a much more important factor than seed infection in producing corn root rot.

In 1925 the part of Block B containing the infected area was sown to millet and in 1926 to oats. Again in 1927 the plot was planted to corn. Twelve rows were planted, each with a different ear, on May 18. Only part of these rows crossed the infected area as mapped in 1924. This planting was duplicated on May 31 and again on June 16. The corn grew normally and developed rapidly. Observations at maturity failed to reveal any typical symptoms of corn root rot. Practically none of the plants went down as in former years and there was no trace whatever of any infected area within which the plants were affected with corn root rot and outside the boundaries of which the plants were not diseased. The failure of corn root rot to cause the plants to go down in 1927 may have been due to the abnormally cool summer or to the lack of virulence of the organisms in the soil producing corn root rot due to the absence of corn growing in the soil for two years. The latter explanation is improbable because soil removed from this plot in the fall of 1926 produced corn plants with typical root rot symptoms in a greenhouse experiment described later. It is more probable that the abnormally cool growing season was responsible for the lack of typical corn root rot development in the field. The average temperature during the month of August, the period during which corn approaches maturity in this section and when corn root rot apparently develops most rapidly, was

70 degrees Fahrenheit or 5.6 degrees below normal. In 1922, 1923 and 1924, the years in which corn root rot did develop on this particular plot, the August temperatures were 1.9, 1.5 and 1.5 degrees respectively above normal. It may be significant that the mean August temperature at Columbia in 1927 was only a fractional part of a degree different from the normal August temperature in Minneapolis which is representative of a section in the upper Mississippi Valley where, according to reports, corn root rot is much less prevalent than in the corn belt proper further south. Dr. I. T. Scott, plant pathologist of the Missouri Experiment Station, reports that only a few specimens suspected of having corn root rot were sent in by corn growers of Missouri in 1927, whereas, dozens of specimens were received by him in 1922, 1923, 1924, 1925, and 1926.

THE EFFECT OF SOIL AND SOIL TREATMENTS ON THE DEVELOPMENT OF CORN ROOT ROT

A number of greenhouse experiments were performed between growing seasons with different soils given various soil treatments. That results from greenhouse experiments can never be fully applied to field conditions was appreciated; nevertheless, it was believed that certain experiments with infected soil could be performed just as satisfactorily in the greenhouse. One great advantage of greenhouse experiments in this case was that corn plants could be grown to maturity during the period between growing seasons. Another advantage was the ability to control to a greater extent the environmental conditions under which the corn plants were grown. The object of the these experiments was to secure some definite information on the organism or soil conditions causing corn root rot on the Missouri Experiment Station field.

Experiment 1.—Corn soil and virgin soil were used in this experiment which was conducted in the greenhouse during the fall and early winter of 1922. Four-gallon glazed pots each with a hole in the bottom for drainage were employed for growing corn plants to maturity. The corn soil in pots 1 and 2 was removed from the base of diseased plants in the so-called infected area on the Field Crops Station field. The virgin soil was secured from two sources: the soil in pots 3 and 4 was surface soil from a pasture near Columbia that had never been plowed; while the soil in pots 5 to 9, inclusive, was excavated subsoil that had weathered about a year. The infected soil, or corn soil, in pots 1 and 2, the pasture virgin soil in pots 3 and 4 and the excavated soil in pot 5 were left untreated. The excavated soil in each of the remaining pots was inoculated with different organisms. A cylinder of soil about 6 inches in diameter by 6 inches deep in pot 6 was mixed uniformly with 50 grams of

a one-month-old rice culture of *Diplodia zeae* grown in a 250cc flask. Pot 7 was similarly inoculated with *Gibberella saubinetii;* pot 8 with *Cephalosporium acremonium*; and pot 9 with *Fusarium moniliforme*. The particular strains of Gibberella, Diplodia, and Fusarium used were obtained from single spores of authenticated cultures. The *Cephalosporium acremonium* had been isolated in 1921 from nearly mature corn stalk internodal tissue with discolored vascular bundles and kept in pure culture in the laboratory.

All pots were planted on September 11, 1922 with disease-free seed. The seed used was considered disease free because 48 kernels from the particular disease-free ear had produced no fungous growth of any kind when isolated aseptically on culture media. As soon as the corn was 6 or 8 inches high, a layer of well-rotted manure about two inches thick was put on top of the soil in each pot to provide a mulch and furnish nutrients for the corn plants. The plants started off well with the exception of No. 7, which was inoculated with *Gibberella saubinetii*. This plant seemed badly injured from the time it came up and finally died at the end of six weeks. The stalk of the dead plant was removed and another disease-free kernel planted in the pot on October 22. A vigorous plant appeared six days later, and it continued to grow normally, appearing just like the other plants except that it was six weeks behind in its development.

All the other plants grew equally well and no difference in the development of above ground parts was noted. All the plants had at least one ear and looked to be about the same size at maturity or 140 days after planting. After cutting the stalk off about four inches from the base of the plant, each pot was emptied carefully on a screen stretched across the top of a box. The dirt was washed carefully from the roots with a hose, great care being taken to preserve all the roots. The roots that grew in infected soil were badly rotted; while the roots from virgin pasture soil were quite healthy, being white in appearance and much better developed. The roots from untreated excavated soil, in pot 5, were likewise quite healthy and fully developed. But the roots from the Diplodia and Cephalosporium inoculated excavated soil, pots 6 and 8, respectively, were badly rotted, resembling those from diseased soil. The roots from the *Fusarium moniliforme* inoculated soil were healthy like those from the untreated soil. The second plant grown in the *Gibberella saubinetii* inoculated soil had a well developed root system, though slightly less in extent than the check, and had many brownish lesions an inch or more in length on the finer roots. The results of this preliminary greenhouse experiment are tabulated in table 22, which appears on page 50.

TABLE 22.—SHOWING EFFECTS OF DIFFERENT SOILS AND SOIL TREATMENTS ON THE DEVELOPMENT OF CORN ROOT ROT

Pot Number	Kind of soil	Condition of roots at maturity
1	Infected	Badly rotted
2	Infected	Badly rotted
3	Virgin pasture	Healthy
4	Virgin pasture	Healthy
5	Virgin excavated (check)	Healthy
6	Virgin excavated (inoculated with Diplodia)	Badly rotted
7	Virgin excavated (inoculated with Gibberella)	Healthy—a few roots slightly rotted
8	Virgin excavated (inoculated with Cephalosporium)	Badly rotted
9	Virgin excavated (inoculated with Fusarium)	Healthy

Drawings of the roots from plant 1 and 3 respectively are reproduced in Plate II. This plate shows the characteristic nature of the root rot disease in a zone beginning one or two inches below the surface of the soil. This feature of the diseased roots being healthy for a distance of an inch or so under the soil is often noticeable on plants growing in the field. Furthermore, the roots in the bottom of the diseased pots also appeared healthy, but all the root tissue in the interior of the soil zone was quite brown to brownish black and about half of it was partially disintegrated. The top dressing of manure on the soil offered an explanation for the healthy condition of the roots near the surface, but subsequent observations in other experiments have shown that this view is untenable.

Many isolations were made from bits of affected root tissue from all the diseased plants. The method used was to wash thoroughly in sterile water a piece of root tissue about one-half inch long, then surface disinfect it by momentary immersion in 95 per cent alcohol, and one minute in 1:1000 mercuric chloride, after which it was washed again in sterile water and placed on a sterile slide. A sterile scalpel was used to cut the root portion into small bits, one or more of which were placed on potato dextrose agar in petri dishes. In most cases a Fusarium either identical with or resembling *Fusarium moniliforme* grew out of the isolated root tissue. Often a Penicillium or a bacterial colony appeared. Neither Diplodia, Gibberella, nor Cephalosporium were isolated from root tissue. However, typical pycnidia of Diplodia formed on the base of the stalk growing in the Diplodia inoculated soil and isolations from stalk tissue gave pure cultures of *Diplodia zeae*. Isolations from the base of the stalk growing in Cephalosporium inoculated soil invariably yielded a Fusarium, usually resembling *Fusarium moniliforme*.

These results quite convincingly indicated the presence in the infected soil of some causal agent producing corn root rot and the absence of such a causal agent in the virgin or uncropped soil. The evidence for the pathogenicity of Diplodia and Cephalosporium seemed inconclusive in view of the fact that neither organism was re-isolated from the rotted roots. No evidence for the pathogenicity of *Fusarium moniliforme* was secured. Inoculation with *Gibberella saubinetii* apparently produced seedling blight in the first planting but no seedling blight and little or no root rot in the second planting. The inoculation results as a whole seemed so inconclusive that plans were made to repeat the experiment but in a much larger way.

Experiment 2.—For this experiment, soil was taken from both inside and outside the infected area in the corn root rot experimental plot on the Experiment Station field. Infected soil was obtained in October, 1923 by removing the central block of soil, about 12 inches square and 5 or 6 inches deep, from a hill of corn containing plants with badly rotted root systems. "Clean" soil was secured in like manner from nearby hills containing plants with apparently healthy root systems. The root systems were deemed healthy if the selected well developed, erect plants were difficult to pull out of the ground and when pulled out had 25 or 30 pounds of soil, if fairly moist, attached to them. None of this clean soil was more than ten yards distant from the infected area of soil in which all the plants succumbed to root rot. Both lots of soil were shoveled into grain bags and removed at once to the greenhouse.

TABLE 23.—SHOWING PLAN OF GREENHOUSE EXPERIMENT NO. 2

Pot number	Kind of soil	Treatment of soil
1A, 1B	Clean	None (check)
2A, 2B	Infected	None (check
3A, 3B	Infected	Sterilized at 15 lbs. pressure for 4 hours.
4A, 4B	Infected	Artificially aerated
5A, 5B	Clean	Inoculated with 200 grams infected soil.
6A, 6B	Clean	Inoculated with 25 grams of the 4 organisms
7A, 7B	Clean	Inoculated with 50 grams rice culture of Gibberella
8A, 8B	Clean	Inoculated with 50 grams rice culture of Diplodia
9A, 9B	Clean	Inoculated with 50 grams rice culture of Fusarium
10A,10B	Clean	Inoculated with 50 grams rice culture of Cephalosporium
11A,11B	Infected	Infected soil half, sand half
12A,12B	Infected	Infected soil three-fourths, sand one-fourth
13A,13B	Infected	Limed—2 tons per A
14A,14B	Infected	Manganese sulphate 50 lbs. per A.

The kinds of seed corn, fungi, inoculation cultures, and pots used in this experiment were the same as in the previous experiment. All pots were set up in duplicate and the kinds of soil and treatments used in each pair of pots are given in Table 23.

The pots to be aerated, those containing manganese sulphate and those containing sand, were included to determine if additional oxygen is an advantage to corn roots growing in infected soil. That the lack of oxygen might be a factor in producing root rot was suggested in the previous experiment when the corn roots in the extreme upper and lower soil zones did not rot appreciably. Additional oxygen was to be given the aerated pots by forcing air into the soil with an air pump. Sand was mixed with infected soil to make it more porous and thus admit more air. Manganese sulphate was added to infected soil in order to increase the oxidative power of the soil. The lime treatments were given in order to change the slightly acid reaction of the soil to slightly alkaline and to supply more available calcium for plant growth.

The corn was planted in all pots on February 2, 1923. Four disease-free kernels were planted in each pot and as the plants came up all but one per pot were removed. On the seventh day after planting plants appeared in all pots except 6B, 10B, 12A, 13A, and 14A and B. These pots were replanted on February 9. Observations made at that time revealed two very sickly appearing plants in pot 6A, which was inoculated with all four organisms. Both plants in sterilized soil were below average in size and were light green in color. All other plants seemed normal and thrifty. By March 4 all the replanted pots had plants up and growing nicely except 10B, which was replanted the second time. One of the two sickly plants left in pot 6A had died by this time and the remaining plant appeared in a wilting condition. Within another ten days, however, this wilted plant seemed to recuperate and began to grow vigorously. Also at this time, March 14, the plants in sterilized soil had acquired a normal dark green color and appeared to be thriving as well as any of the plants. From this time on to maturity there was practically no apparent difference in the stalk growth of any of the plants except those in pots 4A and B, which were artificially aerated.

Aeration of pots 4A and B was attempted by pumping air through a tube extending from near the middle of the pot when filled with soil out through a hole in the bottom. A bicycle pump was used to force air into each pot for five minutes every other day. The manipulation or technique was not considered satisfactory because considerable amounts of soil were lost through the tubes when the pots were watered. The plants showed the effects of the treatment by requiring supports to hold them upright.

All the pots were emptied 130 days after planting and the dirt carefully washed from the roots of the plants as before. The condition of each root system with regard to comparative growth and diseased condition is tabulated in Table 24.

TABLE 24.—SHOWING COMPARATIVE GROWTH AND DISEASED CONDITION OF CORN ROOTS GROWN IN CLEAN AND INFECTED SOIL

Pot number	Kind of soil	Comparative root growth	Condition of roots
1A	Clean (check)	Extensive	Healthy
1B	Clean (check)	Very extensive	Healthy
2A	Infected (check)	Fair	Badly rotted
2B	Infected (check)	Fair	Badly rotted
3A	Infected (sterilized)	Very extensive	Very healthy
3B	Infected (sterilized)	Very extensive	Very healthy
4A	Infected (aerated)	Poor	Badly rotted
4B	Infected (aerated)	Poor	Badly rotted
5A	Clean (infected soil)	Poor	Badly rotted
5B	Clean (infected soil)	Poor	Badly rotted
6A	Clean (4 organisms)	Extensive	Healthy
6B	Clean (4 organisms)	Extensive	Healthy
7A	Clean (Gibberella)	Poor	Healthy
7B	Clean (Gibberella)	Poor	Badly rotted
8A	Clean (Diplodia)	Extensive	Healthy
8B	Clean (Diplodia	Extensive	Healthy
9A	Clean (Fusarium)	Estensive	Healthy
9B	Clean (Fusarium)	Extensive	Healthy
10A	Clean (Cephalosporium)	Extensive	Healthy
10B	Clean (Cephalosporium)	Extensive	Healthy
11A	Infected (one-half sand)	Poor	Badly rotted
11B	Infected (one-half sand)	Poor	Badly rotted
12A	Infected (one-fourth sand)	Poor	Badly rotted
12B	Infected (one-fourth sand)	Poor	Badly rotted
13A	Infected (Limed)	Poor	Badly rotted
13B	Infected (Limed)	Poor	Badly rotted
14A	Infected (Manganese)	Poor	Badly rotted
14B	Infected (Manganese)	Poor	Badly rotted

Without exception the plants grown in untreated diseased soil had badly rotted root systems. Treatments with lime, manganese, and different amounts of sand had no deterrent effect on the development of corn root rot. Sterilization of the infected soil, however, did prevent roots growing in it from becoming diseased. This suggested that sterilization of the soil destroyed the causative agent producing root rot. And the fact that the plants growing in clean soil inoculatd with *Diplodia zeae*, *Fusarium moniliforme*, and *Cephalosporium acremonium*, respectively, produced healthy root systems like those in the clean soil check pots strongly suggested that these organisms were not responsible for the diseased roots in the infected soil. The results from inoculating clean

soil with *Gibberella saubinetii* were contradictory and may be explained in one of three ways: first, the organism may have been dead in the inoculum used in the soil producing a healthy root system; second, the soil producing the diseased root system may have become accidentally contaminated with infected soil by splashing while watering or by other means; and third, the condition of the root system may have been due to after affects of seedling blight due to *Gibberella saubinetii* from which the plant never fully recovered. Either of the last two views seems to the writer more plausible than the first one. It was at this time that the writer was obliged to give up all work on the corn root rot problem; thus no opportunity was afforded for making isolations of the diseased roots to determine the various organisms involved.

At this point in the investigations, although too early to draw any conclusions, it seemed quite obvious that corn root rot, at least as it occurred on the experimental plots, was caused by some unknown soil-borne organism hitherto unassociated with diseased corn roots, stalks or seeds. But two years intervened before further experiments could be performed to determine whether or not this was actually the situation, and if so, to study the nature of the organism involved.

It is significant to note here that before the corn root rot investigational work was resumed in the botany laboratory of the University of Missouri in the fall of 1926, Valleau[119] published a paper in which he reported soil sterilization and inoculation experiments conducted in 1925 which were nearly identical with those just discussed. In greenhouse experiments Valleau used infected soil from a field in which most of the corn plants invariably went down just before maturity. For clean soil he used either virgin soil or sterilized soil. He found no rotting of the corn roots when disease-free seedlings were planted in virgin soil, sterilized infected soil, or in clean soil inoculated with *Diplodia zeae*, *Giberella saubinetii*, *Fusarium moniliforme*, and other species of Fusaria. He did, however, obtain badly rotted root systems from healthy seedlings planted in infected soil and in clean soil inoculated with diseased corn roots. The methods used by Valleau were so very similar to those employed in this investigation that it is possible to compare roughly certain of the results obtained. Table 25 is such a comparison.

These results obtained by Valleau in 1925 are almost identical with those obtained in this laboratory in 1924. The only two exceptions occurred in the soil inoculated with *Diplodia zeae* in one case and with *Gibberella saubinetii* in the other. Valleau believes that the Diplodia inoculated soil in which corn root rot developed was contaminated with infected soil. A similar explanation has been offered by the writer for the root rot occurring in the soil inoculated with *Gibberella saubinetii* in

TABLE 25.—COMPARING RESULTS FROM MISSOURI AND KENTUCKY EXPERIMENTS

Kind of soil used	Condition of corn roots in duplicate pots	
	From Missouri experiments	From Valleau's experiments
Clean	Healthy—healthy	Healthy—healthy
Infected	Badly rotted—badly rotted	Badly rotted–badly rotted
Sterilized Infected	Healthy—healthy	Healthy—healthy
Clean—inoculated with diseased corn roots	Badly rotted—badly rotted	Badly rotted—badly rotted
Clean—inoculated with *Gibberella saubinetii*	Healthy—badly rotted	Healthy—healthy
Clean—inoculated with *Diplodia zeae*	Healthy—healthy	Healthy—badly rotted
Clean—inoculated with *Fusarium moniliforme*	Healthy—healthy	Healthy—healthy

the Missouri experiment. From his results Valleau concluded that the organisms commonly associated with infected seed corn and diseased roots and stalks, namely *Diplodia zeae*, *Gibberella saubinetii*, and *Fusarium moniliforme*, are apparently concerned only as secondary invading organisms. He suggested that true corn root rot is caused by a soil-borne fungus which he was unable to isolate in pure culture. Because he found hyphae and spores of a Pythium-like organism always associated with diseased corn roots, he assumed that fungus to be the cause of corn root rot. Measurements of spores and examinations of hyphae of the organism in diseased corn tissue led Valleau to the belief that the organism producing corn root rot may be closely akin to the Pythium-like organism found by Carpenter[14] to produce root rot of sugar cane in the Hawaiian Islands.

Experiment 3.—The investigational work on the corn root rot problem was resumed in the fall of 1926. Valleau's article, suggesting that corn root rot is caused by a Pythium-like fungus, lent new emphasis to the problem of producing typical corn root rot by means of artificial inoculation. Accordingly, sixteen pairs of 4-gallon pots were filled with different kinds of soil and given different treatments as shown in Table 26.

The so-called "clean" soil was taken from the same locality in the Missouri Experiment Station field from which the clean soil had been secured in 1924 for Experiment 2. This locality was about 10 yards from the edge of the diseased soil area as mapped in 1923. The "infected" soil was removed from the infected soil area. The *Diplodia zeae*, *Gibberella saubinetii*, and *Fusarium moniliforme* used were cultures of the same strains used in the earlier experiments. Fifty grams of approximately one-month-old rice cultures were used for inoculation material. The unidentified species of Fusarium used was secured from many isola-

TABLE 26.—SHOWING PLAN OF GREENHOUSE EXPERIMENT NO. 3

Pot number	Kind of soil	Treatment
9A, 9B	Clean	None (check)
10A, 10B	Clean	Inoculated with Gibberella, Fusarium, and Diplodia
11A, 11B	Clean	Inoculated with Diplodia
12A, 12B	Clean	Inoculated with Gibberella
13A, 13B	Clean	Inoculated with Fusarium
14A, 14B	Clean	Inoculated with 200 grams infected soil
17A, 17B	Clean	Inoculated with 10 grams diseased roots
18A, 18B	Clean	Inoculated with sterilized roots
40A, 40B	Clean	Sterilized—planted with diseased seed
1A, 1B	Infected	None (check)
35A, 35B	Infected	Sterilized
36A, 36B	Infected	Sterilized—inoculated with 10 grams diseased roots
37A, 37B	Infected	Sterilized—inoculated with 10 grams sterilized diseased roots
38A, 38B	Infected	Sterilized—inoculated with unidentified Fusarium
39A, 39B	Infected	Sterilized—planted with diseased seed
41A, 41B	Infected	None—planted with diseased seed

tions of thin razor sections of diseased corn roots. The diseased roots used for inoculation were secured from diseased corn plants and invariably contained oospore-like bodies apparently identical with those described by Valleau in diseased corn roots in Kentucky. Ten grams of such dried diseased root material were used to each inoculated pot. The pots were planted November 22 with kernels from a disease-free ear of Reid's Yellow Dent corn. The seedlings came up fairly uniformly in all the pots, but those in the pots inoculated with rice cultures grew a little more slowly for the first two or three weeks. A month after planting, two plants were dead and the pots were replanted immediately. These plants were, 36A, grown in sterilized infected soil inoculated with diseased roots, and 11A, grown in clean soil inoculated with Diplodia. Plant 11A was killed by ants and a second planting in this soil was also killed by ants which were not found infesting any of the other pots. Plant 36A showed no insect injury whatever, but examinations of the roots revealed a non-septate fungus and occasional oospore-like bodies in the diseased tissue of the cortex. Dozens of attempts to isolate a non-septate fungus from bits of this root tissue failed. Many different fungi were isolated, but a large per cent of the isolations gave Fusarium species. There seemed to be no question but that this plant had been killed by a fungous organism, the identity of which could not be determined.

The remaining plants including the second planting in pot 36A produced tassels and ear shoots, and many produced small ears. It was noticeable that the plants growing in inoculated sterilized soil were just as large as the plants growing in sterilized soil. Notes on the comparative size of the plants taken at maturity showed that only those plants growing in untreated clean soil and in the single pot inoculated with Diplodia were noticeably smaller than the others. The experiment

was terminated 135 days after planting. On April 5, the pots were emptied on a screen, and the soil carefully washed from the roots.

As shown in Table 27, only those plants growing in sterilized soil not inoculated with diseased roots had normal and healthy root systems (see Plate III). The fact that the untreated clean soil produced root rot the same as the untreated infected soil indicated that it was infected with the root rot disease. This was doubtless caused by the natural

TABLE 27.—SHOWING CONDITION AND WEIGHT OF ROOTS OF PLANTS GROWN IN EXPERIMENT 3

Pot number	Kind of soil	Condition of roots	Avg. wt. per root system in grams
9A, 9B	Clean (check)	Both badly rotted	2.2
10A, 10B	Clean (3 organisms)	Both badly rotted	4.7
11A, 11B	Clean (Diplodia)	Both badly rotted	2.2
12A, 12B	Clean (Gibberella)	Both badly rotted	4.1
13A, 13B	Clean (Fusarium)	Both badly rotted	8.1
14A, 14B	Clean (Inoc. inf. soil)	Both badly rotted	4.2
17A, 17B	Clean (Inoc. dis. roots)	Both badly rotted	6.3
18A, 18B	Clean (Inoc. ster. roots)	Both badly rotted	3.6
40A, 40B	Clean (Ster. dis. seed)	Both healthy	37.8
1A, 1B	Infected (check)	Both badly rotted	6.0
35A, 35B	Infected (Sterilized)	Both healthy	71.6
36A, 36B	Infected (Sterilized-inoc. dis. roots)	Both badly rotted	8.8
37A, 37B	Infected (Sterilized-inoc. ster. diseased roots)	Both healthy	22.4
38A, 34B	Infected (Fusarium)	Both healthy	30.7
39A, 39B	Infected (Sterilized dis. seed)	Both healthy	46.7
41A, 41B	Infected (Dis. seed)	Both badly rotted	3.9

spreading of the disease in the field during 1924, 1925, and 1926. This might easily have occurred when the field was plowed and harrowed in 1925 and again in the spring of 1926. It will be remembered that the so-called clean soil for this experiment was secured from a place that produced corn plants with healthy roots in 1923 which was only ten yards from the edge of the infected soil area. If it is assumed that the root rot in 1923 was produced by a soil-borne fungus, it would seem strange indeed if all soil within ten yards of the boundary of the infected area had not become infected during tillage operations by October, 1926. In fact, one of the reasons for using the "clean" soil was to determine whether or not it had become infected since 1923.

The root systems of the corn plants grown in "clean" soil inoculated with Diplodia, Gibberella, and Fusarium showed no more root rot than the root systems of plants grown in uninoculated "clean" soil, showing that the effect of these organisms was negligible compared with infected soil in causing corn root rot. Likewise the unidentified species of Fusarium produced no rotting of the roots in the sterilized infected soil. But

the plants growing in sterilized infected soil inoculated with 10 grams of diseased corn roots had badly rotted root systems, identical in appearance with those in infected soil (see plates IV and V). Neither of the sterilized soils inoculated with sterilized roots or planted with diseased seed produced plants showing any sign of root rot. This demonstrates the presence of the root rot organism, or agent if not an organism, in diseased corn roots; and further, that this organism or agent may be destroyed by sterilization.*

Experiment 4.—A similar experiment was started December 1, 1926 when five pairs of pots were set up as follows: two pots with sod soil, used as check; two with sod soil inoculated with 200 grams infected soil; two with sod soil inoculated with 10 grams of diseased corn roots; two with sod soil containing 10 grams of sterilized diseased roots; and two with infected soil used as check. All these plants grew normally, but at maturity the plants in the sod soil inoculated with diseased roots were approximately 12 inches shorter and ¼ inch less in diameter of stalk than the other plants. The results giving the weight and condition of the roots are given in Table 28.

TABLE 28.—SHOWING CONDITION AND WEIGHT OF ROOTS OF CORN PLANTS GROWN IN UNTREATED SOD SOIL AND INOCULATED SOD SOIL—GREENHOUSE EXPERIMENT 4

Pot number	Kind of soil	Condition of roots	Avg. wt. per root system in grams
19A, 19B	Sod (check)	Both healthy	19.1
20A, 20B	Sod (inf. soil)	Both badly rotted	5.25
22A, 22B	Sod (inf. roots)	Both badly rotted	4.45
23A, 23B	Sod (ster. inf. roots)	Both badly rotted	6.2
2A, 2B	Infected (check)	Both badly rotted	5.9

All the root systems showed root rot except those growing in the untreated sod soil. One of the latter plants did not produce as extensive a root system as the duplicate plant, but there were no visible lesions on the roots. The root systems of plants 23A and B, grown in sod soil containing sterilized infected roots, showed nearly as much root rot as the plants grown in sod soil inoculated with infected roots. This result was probably caused by accidental contaminations, inasmuch as the root systems grown in sterilized soil containing sterilized infected roots in Experiment 3 were quite healthy. These results indicate that the sod soil does not contain the root rot producing organism or agent.

Experiment 5.—In this experiment some of the same infected soil was used as in Experiments 3 and 4. Duplicate pots of soil were given different fertilizer and lime treatments and planted to disease-free seed.

*The sterilized soil in this experiment was autoclaved for 15 minutes at 15 pounds pressure, then spread out in a layer about 2 inches thick on tables for a week before putting it in sterilized pots. Every precaution was taken to prevent the soil becoming contaminated with corn disease organisms or with infected soil during the process.

Potassium chloride, acid phosphate, and ground limestone applications were made at the rate of 200 pounds, 300 pounds, and 6000 pounds per acre, respectively. At the same rates, potassium chloride was also applied in combination with acid phosphate, limestone and with acid phosphate and limestone. The plants grew normally and at maturity they looked very much alike. Table 29 gives the results of this experiment.

TABLE 29.—SHOWING THE EFFECT OF DIFFERENT LIME AND FERTILIZER TREATMENTS ON THE DEVELOPMENT OF CORN ROOT ROT IN INFECTED SOIL

Pot number	Soil treatment, lbs. per acre	Condition of roots	Avg. wt. per root system in gram
3A, 3B	None (check)	Badly rotted	4.2
24A, 24B	Potash	Badly rotted	5.9
25A, 25B	Phosphate	Badly rotted	5.7
26A, 26B	Limestone	Badly rotted	5.7
27A, 27B	Potash and limestone	Badly rotted	7.1
28A, 28B	Potash and phosphate	Badly rotted	6.4
29A, 29B	Potash, limestone, and phosphate	Badly rotted	8.5
34A, 34B	None (diseased seed)	Badly rotted	5.2

It will be noted in the table above that there was little difference in the weights of the roots grown in treated or untreated soil. Plants 34A and B, from grains infected with Diplodia and Fusarium, showed no more root rot than the check plants.

The root systems of plants 29A and B, grown in infected soil treated with limestone, potash, and phosphate were slightly heavier and more extensively developed than the others, but they were just as badly rotted. This slight increase in growth in heavily fertilized infected soil is to be expected, inasmuch as Valleau and co-workers[119] reported an increased root growth from fertilized infected soil compared with untreated infected soil. The character of the root development of most of the plants in this experiment was typical of corn roots grown in pots containing infected soil in that less rotting occurred in the bottom one or two inches of soil and in the top inch of soil. The writer is unable to state the reason or reasons for this condition. The comparatively healthy mass of fine roots growing in the bottom of pots containing infected soil was always found except in those cases in which the roots were so severely rooted in the middle of the pot that the translocation of food to the lower roots was apparently cut off. In such cases there was evidence to show that the fine roots had developed earlier in the life of the plant. The conclusion drawn from the results of this experiment was that the application of neither potash, phosphate, nor limestone to the soil used reduced the amount of root rot in corn plants grown in it.

At the time the corn in Experiments 3, 4, and 5 was planted, disease-free seed was planted in 10 other pots containing infected soil. These plants were removed and the soil washed from the roots at different stages of growth to determine when the root rot disease made its appearance. Very little rotting was found on roots grown for 30 days, 60 days, 90 days, and 100 days. Observations of roots more than 100 days old indicated that root rot developed very rapidly from about the 100th day until maturity. There was evidence, however, that many roots had been killed as they started to grow during the first 100 days since the roots that did develop were much less numerous than in uninfected soil, though apparently not diseased. When sections of these diseased roots were examined with the microscope mycelium and spores of a Pythium-like fungus which resembled those described by Valleau and co-workers[119] were invariably found. Many other kinds of mycelia and spores were also present in the roots. The writer succeeded in isolating a Pythium-like fungus from diseased roots of a 103-day-old corn plant. Evidence to show the relation of this organism to root rot will be discussed in the next few pages of this paper.

THE PROBABLE CAUSE OF CORN ROOT ROT

Corn root rot as it occurs in Missouri probably is caused by a Pythium-like organism similar to the fungus found by Carpenter[14] to produce a root rot of sugar cane in Hawaii. The organism found by the writer in rotted corn roots is undoubtedly identical with the one Valleau and co-workers[119] found invariably present in diseased corn roots in Kentucky. However, they were not able to isolate it in pure culture. Measurements of oospores of the organism growing in pure culture on corn roots in prune juice are the same as oospores of *Phytophthora cactorum* and the two kinds of oospores look just alike. This suggests with practical certainty that Clinton[16] saw oospores of this organism when he reported the occurrence of *Phytophthora cactorum* in corn stalks, the roots of which were severely infected with root rot.

After reading Valleau's paper in September, 1926, the writer began at once to examine diseased roots of corn plants growing on the Station field for the Pythium-like oospores and mycelium. All of the diseased specimens examined revealed these spores and mycelium as well as many other kinds of spores and mycelia, the latter principally septate. Numerous unsuccessful efforts were made to isolate the Pythium-like organism. In the earlier isolation work the root tissue containing oospores was surface sterilized in alcohol and mercuric chloride, washed in sterile water, and then thinly sectioned with a razor and placed aseptically in the culture media.

In nearly every case the unidentified species of Fusarium, mentioned in Experiment 3, was obtained either in pure culture or in combination with other fungi and sometimes bacteria. Many kinds of culture media were tried, including cornmeal, rice, apple, cornmeal agar, potato dextrose agar, prune agar, prune juice, nutrient bouillon agar, and others. Fresh diseased material was obtained all winter from plants growing in the greenhouse in infected soil, but all efforts to isolate the Pythium-like organism were fruitless. A paper by Hartley[39] suggested that often filamentous fungi are killed when tissue containing them is sterilized with mercuric chloride; so isolations were made from diseased tissue washed thoroughly in sterile water, using a weak prune juice agar for the culture medium.* The bits of isolated tissue were examined under the low power objective every few hours for the appearance of non-septate hyphae. When such hyphae appeared the ends were clipped and isolated on another culture plate. From about 120 isolations from root tissue, four Pythium-like fungi were obtained in pure culture. Three of these proved to be identical while the fourth one is apparently different only in that it produced oogonia and chlamydospores abundantly on potato dextrose agar. It has been difficult to find a medium on which the others produce fruiting bodies. However, after more than 20 different media were tried, it has been found that the organism fruits abundantly on sterilized corn roots in prune juice. In such a medium oospores and chlamydospores are produced in countless numbers. Fair oospore production was secured on sterile carrot plugs in test tubes.

Little opportunity has been afforded to study the taxonomy of the organism in order to dermine definitely its identity. In a pure culture growing on corn roots in prune juice, mycelium, oogonia, and oospores are produced abundantly and chlamydospores less abundantly. The hyphae are non-septate and vary considerably in diameter due to peculiar swellings occurring at intervals. Ordinary hyphae are 2 to 4 microns in diameter, while the swollen places vary in diameter up to 20 microns (see Plate VI A). Oogonia are spherical averaging 30 to 32 microns in diameter. Oospores average 28 to 30 microns in diameter and almost completely fill the oogonial activty (see Plate VI, B, C, and D). The type of antheridium shown in Plate VI suggests that this organism belongs to the genus Pythium. No zoosporangia nor zoospores have been observed. It is practically certain, however, that the organism is not *Phytophthora cactorum*, a species of a closely related genus, since C. M. Tucker, a graduate student at the University of Missouri in 1926-1927, found that the corn root rot organism did not attack inoculated potato

*The culture medium was made by adding the juice of 50 grams of well cooked prunes and 20 grams of agar to water to make one liter.

tuber tissue, while similar inoculations with *Phytophthora cactorum* produced rotting of the potato.

No conclusive inoculation trials have been carried out to prove the pathogenicity of the Pythium-like organism in producing typical corn root rot. Results of preliminary inoculation trials, however, are very suggestive. Sterilized sand pots were each inoculated with five grams of a corn meal culture of the Pythium-like fungus and immediately planted with disease-free corn seed. The plants in the uninoculated sand came up a day sooner and grew vigorously. The inoculated seedlings, on the other hand, grew much more slowly at first and after about four days looked as if they were about to die. Examinations made at this time revealed the inoculated seedlings with badly rotted mesocotyls and temporary roots; while the check plants were quite healthy (see plant VII). The Pythium-like organism was readily isolated in pure culture from certain portions of the diseased tissue. The organism was seldom isolated from tissue which contained actively growing mycelium as shown in Plate VIII A. A similar difficulty in obtaining a fungus in pure culture was encountered by Jones and Drechsler[67] in isolating a root rot producing organism, *Aphanomyces euteiches* (n. sp.), from diseased pea roots. Other portions of these young diseased roots produced the organism readily.

Additional evidence to show that the particular corn root rot disease affecting plants grown on the Station field is the same as that affecting corn in other parts of the State was secured from two sources. Archer[3], who conducted a disease survey of Missouri in 1926 for the United States Department of Agriculture, found corn root rot prevalent in all parts of Missouri as shown in Fig. 5. Many of his observations were made in July, August, and September when he determined the amount of infection largely by the percentage of down stalks with rotted root systems. These down plants resembled those on the corn root rot plots at Columbia in that the roots gave way about two inches below the surface of the soil, letting the plant fall to the ground with the ends of the rotted roots exposed (see Plate I). The other source of evidence was secured from a preliminary experiment with disease-free corn seed grown in 4-gallon sand pots each containing 200 grams of corn soil from different parts of Missouri. These corn soil samples, which came from corn fields thought to be diseased with corn root rot in 1926, were sent to the writer by the County Agents of DeKalb, Callaway, Linn, Pettis, Atchison, and Cole counties. The corn plants were removed from the sand pots containing corn soil 102 days after planting. All the root systems grown in inoculated sand pots were rotted, while those grown in sand alone were not rotted. Roots of plants grown in sand inoculated with

corn soil from DeKalb and Cole counties were badly rotted, notwithstanding the fact that the plants were little more than 100 days old. Only a part of the roots of each of the remaining root systems showed root rot. When these rotted roots were examined microscopically hyphae and spores of the Pythium-like organism were always found (see Plate VIII).

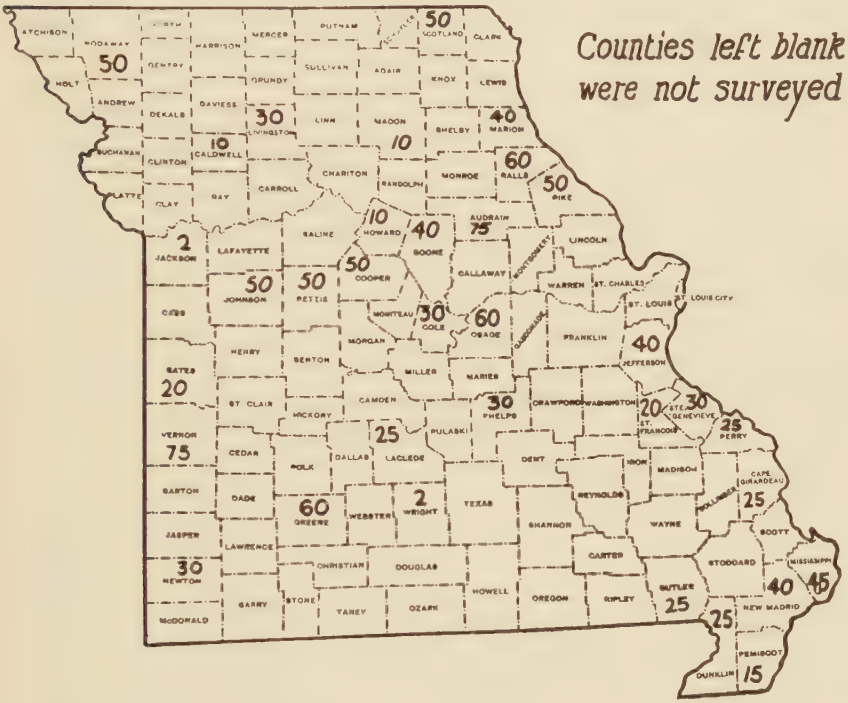

Fig. 5.—Showing percentage of corn root rot infection, by counties in 1926.

Preliminary field experiments carried on during the summer of 1927 failed to give conclusive evidence concerning the pathogenicity of the Pythium-like organism. For these experiments a sod plot on the west end of Block C was used that had been in grass at least 20 years.

The first plantings were made April 16 with disease-free seed in the greenhouse in 5-inch pots containing sterilized sand. A number of pots were left uninoculated for checks, five pots were inoculated at planting time with 25 grams each of a rice culture of the Pythium-like organism 22; five with a similar culture 35; five with *Diplodia zeae*; five with *Fusarium moniliforme* and five with *Gibberella saubinetii*. On May 16 the plants were transplanted in the sod plot in adjacent rows. All the inoculated seedlings were stunted compared with the checks; but only one, which

was inoculated with *Fusarium moniliforme*, died in the field after transplanting. Within a month after transplanting, the seedlings inoculated with Diplodia, Fusarium and Gibberella seemed to recover from the earlier effects of the inoculation and appeared as vigorous and thrifty as the checks. The two rows inoculated with the Pythium-like organisms, however, continued backward and non-vigorous throughout the growing season. One of these plants reached a height of only three feet and produced a small ear on a shoot more than a foot long. Another one of these plants produced a small ear on a shoot that grew out of the first node above the surface of the ground. Of the ten hills inoculated with the Pythium-like organisms all but one went down in September with badly rotted root systems. The plant that remained standing was only three feet high and bore a small ear but when examined at maturity had a badly rotted root system. All of the plants inoculated with Diplodia appeared normal at maturity. The plants were erect with the exception of one, which was leaning, and all had good root systems. All the plants inoculated with *Fusarium moniliforme* reached normal size, stood erect at maturity and had good root systems. One plant inoculated with *Gibberella saubinetii* went down in September but had a well developed and healthy appearing root system at maturity. All other plants inoculated with this organism were normal and had good root systems. The Pythium-like organism was isolated in pure cultures only from bits of diseased root tissue from plants that had been inoculated with Pythium. The writer was unable to isolate this organism from bits of root tissue of the check plants. These results showed very conclusively the pathogenicity of the Pythium-like organisms. But later plantings gave no such indications. Another series similar to the first planting was planted in the sod plot on May 18, except the corn was planted in the hill at the time the inoculations were made. Still another planting was made May 31. Observations made on these later plantings during the growing season and at maturity when the root systems were dug up and washed out showed that the inoculation had no apparent effect on the corn plants. The fact that the later plantings did not produce characteristic root rot symptoms may have been due to a different method of inoculation than was used in the first planting. Another reason for the absence of root rot in the later plantings may have been the unusual weather conditions that prevailed during the growing season. This suggestion is reinforced by the fact that practically no root rot occurred in the infected area of Block B. No planting was made on this plot April 16 at the time the first inoculated series was planted but a series of 12 rows was planted on May 18, another from the same lot of seed on May 31, and another on June 16. All except a few rows of the first series extended across the infected area of soil as it was mapped in

1923 and 1924. It may be that corn root rot failed to occur in this infected area because of the unusual weather conditions since corn planted at the same time in inoculated sod soil did not develop root rot symptoms. If this assumption is correct we would have to further assume that the seedlings employed in the first planting developed under more favorable conditions for infection with the corn root rot organisms.

It is well to point out here that further work on the problem is needed to determine whether typical corn root rot may be produced by growing disease-free seedlings to maturity under field conditions in uninfected soil inoculated with the Pythium-like organism. Furthermore, it will be necessary to isolate the Pythium-like organism from diseased corn roots grown in many other parts of Missouri in order to show that corn root rot, as it occurs on the Station field at Columbia, is typical of corn root rot that occurs elsewhere in the State.

From the results of investigations reported in this paper showing that reductions in yield from planting heavily infected seed corn are due to seedling blight and not to root rot; that root rot does not develop in corn plants grown in uninfected soil inoculated with either of the four most common seed-borne organisms; that corn root rot does develop in plants grown in uninfected soil inoculated with diseased corn root containing spores and mycelium of a Pythium-like fungus; and that this Pythium-like organism may be easily re-isolated from young corn plants after being inoculated and becoming infected with a pure culture of the fungus, it is believed that corn root rot in Missouri is caused by a Pythium-like fungus similar to the one found by Carpenter to be the cause of root rot of sugar cane in Hawaii.

SUMMARY

1. Corn root rot is a disease primarily affecting roots of the corn plant causing them to rot before the plant matures. Seedling blight is a disease affecting seedlings only, causing them to die or become stunted in their development. Corn ear rot and corn stalk rot are diseases resulting from the attacks of the fungi that produce seedling blight and probably some that do not produce seedling blight.

2. The tip one-fifth of every internally infected corn kernel contains one or more fungi, while the remaining parts of the kernel may or may not be infected.

3. Most of the kernels on nearly every ear of corn grown in Missouri are internally infected with either *Fusarium moniliforme*, *Diplodia zeae*, or *Cephalosporium acremonium*. Ears with kernels infected with *Gibberella saubinetii* are extremely rare.

4. Certain physical ear and kernel characters are reliable guides in selecting seed corn that is comparatively free from fungous infection.

This method of selecting is just as effective and more practical than the germinator method for eliminating badly diseased ears.

5. Treating badly diseased corn seed with alcohol and mercuric chloride for one hour markedly reduces the amount of seedling blight when the seed is grown for eight or nine days on the germinator, but the treatment is not effective in reducing the corn root rot in the field and reduces the stand materially.

6. There is no difference in yield from planting heavily infected and lightly infected seed in Missouri, provided the field stand from each lot of seed is the same.

7. Reduced yields from planting diseased seed are due entirely to seedling blight and its after effects.

8. Field experiments conducted on soil infected with root rot gave no promise of controlling the disease by planting disease-free seed or by treating the soil with liberal applications of phosphate, limestone, or phosphate and limestone.

9. Corn root rot does not occur when disease-free corn seedlings are grown in virgin soil nor in infected soil if sterilized.

10. Uninfected soil inoculated with *Diplodia zeae*, *Gibberella saubinetii*, *Fusarium moniliforme*, and *Cephalosporium acremonium* produces a certain amount of seedling blight, under greenhouse conditions, but no root rot in the plants that survive.

11. Disease-free seedlings grown in sterilized infected soil inoculated with corn roots diseased with root rot develop into average sized plants in the greenhouse, but have badly rotted root systems typical of roots diseased with corn root rot.

12. A Pythium-like fungus was isolated from diseased corn roots growing in infected soil. The roots of young corn plants were successfully inoculated with this organism, which was re-isolated in pure culture from the inoculated roots.

13. Corn planted and inoculated with the Pythium-like organism in the field at different dates during the spring of 1927 developed typical corn root rot symptoms from the first planting made April 16, but not from later plantings. It is suggested that the unusually low temperature of 5.6 degrees below normal during August may have been a factor in preventing development of root rot in the later corn.

14. Corn root rot in Missouri is probably caused by a soil-borne Pythium-like fungus.

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BIOGRAPHY

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Plate I.—(A) Showing manner in which roots are exposed when nearly mature corn plants go down with corn root rot. This photograph was taken on the Missouri Experiment Station field crops field in 1923.

Plate II.—Reproduction of an artist's drawing showing (left) a healthy corn root system, and (right) a diseased corn root system.

Plate III.—(35A) Root system of corn plant grown in sterilized infected soil (check). Weight 126 grams.

Plate IV.– (1A, 1B) Root systems of corn plants grown in infected soil (check). Average weight 6.0 grams.

Plate V.—(36B) Root system from plant grown in sterilized infected soil inoculated with diseased corn roots. Weight 8.1 grams.

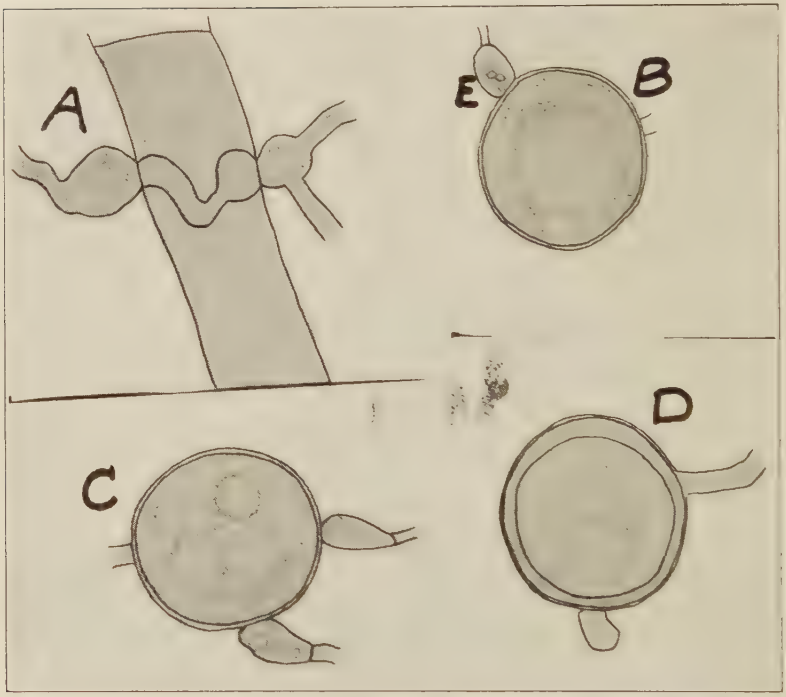


Plate VI.—Camera lucida drawings of spores, hyphae, and antheridia of the Pythium-like fungus isolated from diseased corn roots.

A. A hypha growing in pure culture in a corn root. Note swollen places where hypha penetrates host cell wall.

B. C. Showing oogonia with antheridia (E) attached. Note oogonial stalk and protoplasmic content of antheridia.

D. Fully developed cospore which completely fills oogonial cavity. Note empty antheridium.

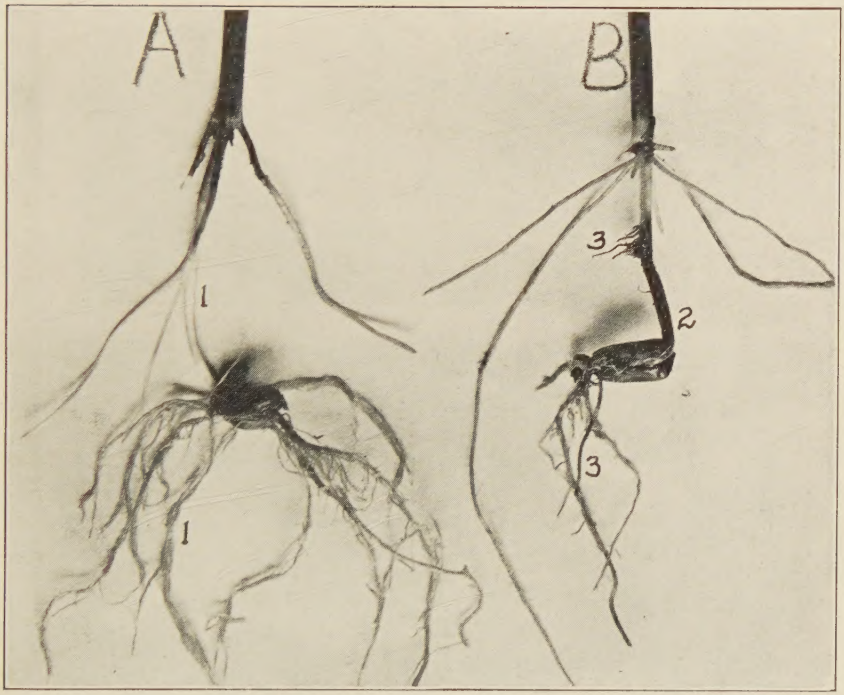
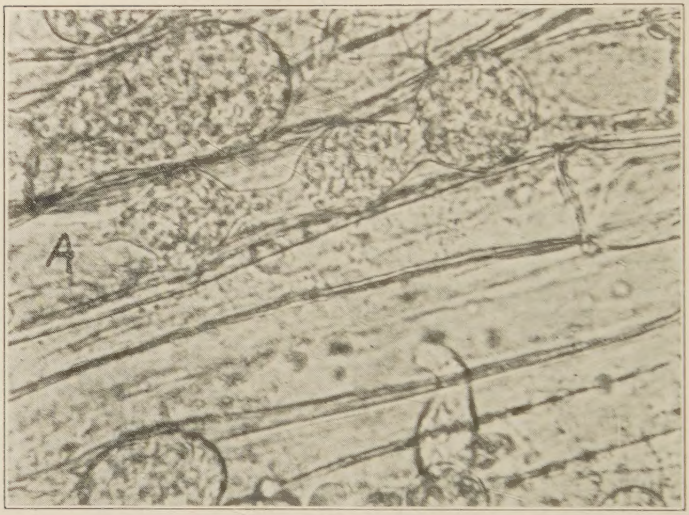


Plate VII.—A. Corn seedling grown in uninoculated sterilized sand. Note healthy mesocotyl (1) and roots.

B. Corn seedling grown in sterilized sand inoculated with the Pythium-like fungus. Note badly rotted mesocotyl (2), and roots. (3).



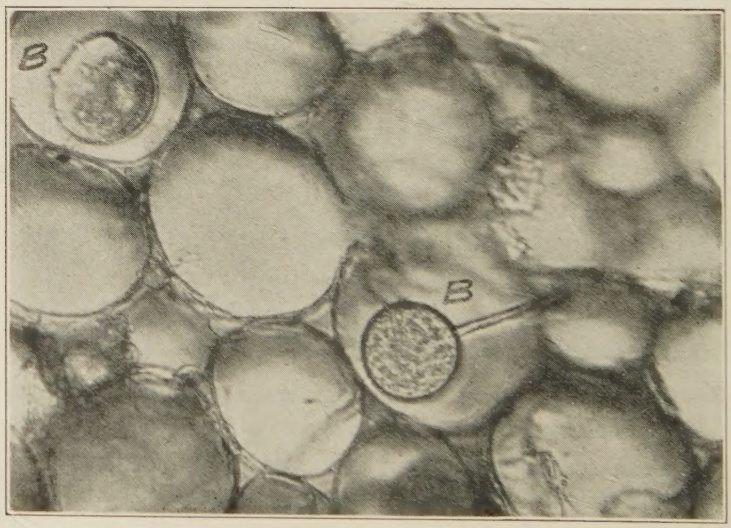


Plate VIII. A. Microphotograph of a crushed corn rootlet of an inoculated plant grown in sand. x 600.

B. Microphotograph of spores of Pythium-like fungus in diseased corn roots of mature plants grown on Station field, 1926. x 540.